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Peptidoglycan Types of Bacterial Cell Walls and their Taxonomic Implications

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INTRODUCTION

The cell wall is the basis for several classical taxonomic characteristics. It not only contributes to the shape of the bacterium but is also responsible for the different stainability of a cell, for most of the serological behavior, and for phage adsorption.

One of the basic markers for the differentiation of bacteria is the so-called Gram reaction. The gram-positive bacteria are distinguished by their ability to hold back the dye-iodine complex, whereas the gram-negative organisms are decolorized after treatment with alcohol. The mechanism of the Gram reaction has not yet been unraveled, but Salton (326) favors an involvement of the cell wall and has suggested a permeability difference between the cell walls of gram-positive and gram-negative bacteria as the basis for the Gram reaction. This is in agreement with the findings that the positive or negative response to this reaction is reflected in the different ultrastructure of the cell wall. The cell wall of a gram-positive bacterium shows in profile one thick and more or less homogenous layer, whereas the profile of the cell wall of a gram-negative bacterium is remarkably complex and consists of several layers. A number of excellent monographs and reviews have recently appeared (see references 57, 89, 118, and 269 for more detailed information).

The polymers making up the cell walls are chemically quite different in these two groups of bacteria. The gram negatives contain as major components lipopolysaccharide, lipoprotein, and relatively little peptidoglycan (less than 10% of the total cell wall) in their cell walls, whereas the walls of gram positives are mainly composed of peptidoglycan (usually 30–70% of the total cell wall), polysaccharides or teichoic acid (or both), or teichuronic acid.

The peptidoglycan is the only cell wall polymer common to both gram-negative and gram-positive bacteria. It has also been found among blue-green algae (96, 102, 142). Thus peptidoglycan is a cell wall component of all procaryotic organisms. There are only a few halophilic bacteria, such as *Halobacterium halobium* (326, 363) and *Micrococcus morrhuae* (177), which lack peptidoglycan. The composition and structure of the peptidoglycan seem to be rather constant among gram negatives, but there is great variation among gram positives. Numerous reviews have recently appeared on the structure and biosynthesis of the peptidoglycan (109, 117, 237, 279, 284, 333, 354, 381, 386, 403, 426). Since that time our knowledge about the diversity of the chemical structure of the peptidoglycan has increased. Moreover, a high percentage of all known genera and of many different species of bacteria has been examined, and it now seems worthwhile to focus on the correlation of peptidoglycan structure and taxonomy. Therefore, in the first part of this review a classification of the known peptidoglycan types based on their mode of cross-linkage will be suggested. In the second part, the distribution of the various peptidoglycan types within the bacterial kingdom will be shown and their taxonomic significance will be evaluated.

The following are abbreviations and uncommon amino acids and amino sugars used throughout the paper. Abbreviations: ATCC, American Type Culture Collection, Rockville, Md., U.S.A.; CCM, Czechoslovak Collection of Microorganisms, J. E. Purkyne University, Brno, Czechoslovakia; Kiel, Streptokokkenzentrale im Institut für Milchhygiene der Bundesanstalt für Milchwirtschaft, Kiel, BRD; NCDO, National Collection of Dairy Organisms, Reading, England; NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland; NCPP, National Collection of Plant Pathogenic Bacteria, Harpenden, England; NCTC, National Collection of Type Cultures, London, England. Amino acids and amino sugars: $GlcNH_2$ or G, glucosamine; Dab, diaminobutyric acid; m-Dpm, *meso*-diaminopimelic acid; Hsr, homoserine; HyDpm, hydroxydiaminopimelic acid; Hyg, threo-3-hydroxyglutamic acid; HyLys, hydroxylysine; Mur or M, muramic acid; Orn, ornithine.

DIFFERENT PRIMARY STRUCTURES OF THE PEPTIDOGLYCAN

General Structures

The rigidity of the bacterial cell wall is due to a huge macromolecule (403) containing acylated amino sugars and three to six different amino acids. This polymer has been called by a variety of names: "basal structure" (425), "mucopeptide" (232), "glycopeptide" (365), "glycosaminopeptide" (326), "murein" (403), and "peptidoglycan" (369). We have usually prefered to use the name "murein," which was introduced by Weidel and Pelzer (403) in analogy to "protein." But there is now a general agreement that "peptidoglycan" is the better term, since it describes the chemical nature of this polymer most exactly. Peptidoglycan is a heteropolymer built out of glycan strands cross-linked through short peptides. The general features of the two peptidoglycan constituents, the glycan and the peptide moiety, will be separately discussed first.

Glycan strands. The glycan moiety of the peptidoglycan is remarkably uniform. It is usually made up of alternating β-1,4-linked *N*-acetylglucosamine and *N*-acetyl muramic acid residues (109, 111). The latter amino sugar, found only in bacteria and blue-green algae, was

first discovered by Strange and Dark (364). Further studies have shown that it is the 3-*O*-D-lactic acid ether of glucosamine (365, 384) (Fig. 1). The glycan reveals only few variations, such as acetylation or phosphorylation of the muramyl 6-hydroxyl groups (9, 109, 224) and the occasional absence of a peptide or *N*-acetyl substituent (10, 109). Recent studies on the peptidoglycan of bacterial spores have indicated that muramic acid residues can be present in the muramic lactam form, a sugar not previously found in nature and, hence, a unique spore constituent (401). Among mycobacteria (3, 25), *Nocardia kirovani* (130) and *Micromonospora* (398), muramic acid does not occur as *N*-acetyl, but as the *N*-glycolyl derivative. Here the amino group in position 2 is not substituted by an acetyl group (—$COCH_3$) but by a glycolyl group (—$COCH_2OH$). Studies on a wide variety of gram-positive and gram-negative bacteria indicated that only glucomuramic acid occurs in the wall: galactomuramic acid has not been found so far (413, 414).

The chain length of the glycans has been discussed in detail by Ghuysen (109). The glycans are polydisperse and thus only average figures can be given. In different organisms the average chain length varies between 10 and 65 disaccharide units (109, 148). Although there is a relationship between the cell shape and average chain length of the glycan in some special cases (210), there is no evidence for a general correlation (204, 402).

Peptide moiety. The peptide moiety is bound through its N terminus to the carboxyl group of muramic acid and contains alternating L and D amino acids. The occurrence of amino acids with the D configuration is a typical feature of the peptidoglycan. A fragment of the primary structure of a peptidoglycan is shown in Fig. 1. Usually L-alanine is bound to muramic acid, followed by D-glutamic acid, which is linked by its γ-carboxyl group to an L-diamino acid, and finally D-alanine is attached to the diamino acid. In some cases the α-carboxyl group of glutamic acid is substituted and an additional D-alanine is found at the C terminus. This part of the peptide moiety is called the peptide subunit (109). The amino group of the L-diamino acid, not bound in the peptide subunit, forms a peptide linkage to the C terminal D-alanine of an adjacent peptide subunit or is substituted through an interpeptide bridge. Thus, the peptide moiety of the peptidoglycan can only consist of the peptide subunit or of the peptide subunit and an interpeptide bridge. The interpeptide bridges cross-link the peptide subunits and extend

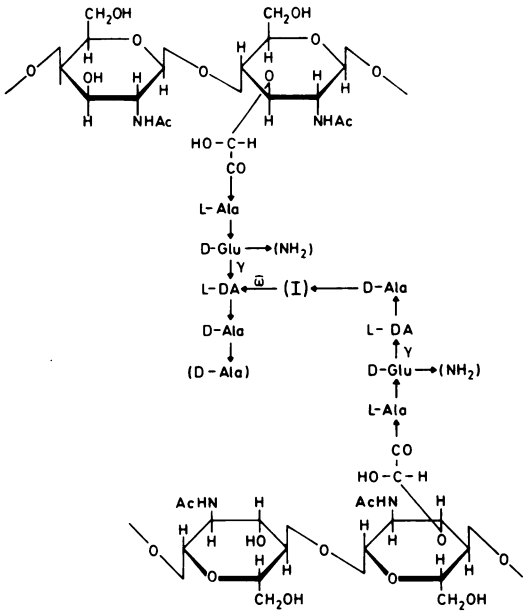

FIG. 1. *Fragment of the primary structure of a typical peptidoglycan. (For the sake of simplicity we do not use the conventional representation as in original publications; we think that the simple scheme which we employ in this paper is more easily comprehensible for the less chemically oriented reader.) Abbreviations:* L-DA, L-*diamino acid; I, interpeptide bridge; Ac, acetyl or in a very few cases glycolyl; ω, ω-amino group of* L-*diamino acid; substituents in parentheses may be absent.*

usually from the ω-amino group of the diamino acid of one peptide subunit to the D-Ala carboxyl group of another peptide subunit. In a minority of cases it extends from the α-carboxyl group of D-glutamic acid to the carboxyl group of D-alanine of another peptide subunit. The interpeptide bridges show great variation in their chemical composition and will be discussed later.

Determination of the Amino Acid Sequence

The amino acid sequence (primary structure) of the peptidoglycan can be determined either by the use of enzymes or by chemical methods.

Enzymatic procedure. The first known amino acid sequence of a peptidoglycan was established by the pioneering work of Weidel and his school (403). They used autolytic enzymes and lysozyme to hydrolyze the peptidoglycan of *Escherichia coli*, isolated the fragments, and determined their structure chemically. Independently of Weidel's group, Ghuysen and co-workers used muralytic enzymes to elucidate the primary structure of the peptido-

glycan of gram-positive bacteria. The methods applied are described in detail in recent reviews (109, 117, 367).

Chemical method. When it was known that the glycan moiety varies very little and that the peptide moiety is built from a very limited number of amino acids, it was possible to use a combination of purely chemical methods to elucidate the primary structure of the peptide moiety of the many different types of peptidoglycan. This "chemical method" was introduced by Schleifer and Kandler (336) and has been used extensively by their group since then. Table 1 summarizes the main steps for the determination of the amino acid sequence. Quantitative amino acid determination was performed with an amino acid analyzer. The configuration of the amino acids was determined either enzymatically (216, 271, 336) or by measuring the optical rotatory dispersion of the 2,4-dinitrophenyl (DNP) derivatives (50, 176). The C- and N-terminal amino acids of the peptidoglycan and of peptides were determined by hydrazinolysis (47, 176) and by dinitrophenylation (114, 310).

The determination of the N-terminal amino acid of the undegraded peptidoglycan is a good indication of the N-terminus of the interpeptide bridge. The cross-linkage of the peptide subunits is usually incomplete. Therefore, a certain percentage of the interpeptide bridges carry a free N-terminal amino group, and, by means of dinitrophenylation of the undegraded cell walls, the N-terminal amino acid of the interpeptide bridge can be established. The dinitrophenylated amino acids were identified by paper chromatography (271, 336) or by thin-layer chromatography on silica gel (39).

The most important step of this chemical method is the isolation and identification of oligopeptides after partial acid hydrolysis of the cell walls. Different kinds of cell wall preparations were used. All of the cell wall preparations were first purified by digestion with trypsin. In some cases further removal of nonpeptidoglycan material was achieved by extraction with cold or hot trichloroacetic acid (20, 21, 110) and with hot formamide (287). These cell wall preparations were treated with 4 N HCl at 100 C for different times. Usually a very short (10 to 20 min) and a somewhat longer (45 to 60 min) hydrolysis were chosen since the stabilities of the various peptides are quite different. Figure 2 shows the kinetics of the release of some peptides during the hydrolysis of the cell walls of *Sarcina lutea* ATCC 383.

TABLE 1. *Procedures for the determination of the amino acid sequence of peptidoglycans*

(i) Quantitative determination of the amino acids and amino sugars of a pure cell wall preparation. Determination of the configuration of the amino acids.
(ii) Determination of N- and C-terminal amino acids in intact cell walls.
(iii) Isolation and identification of oligopeptides from partial acid hydrolysates of cell walls.
(iv) Isolation and identification of peptidoglycan precursors.
(v) Isolation and identification of muropeptides from a lysozyme lysate of cell walls.

Two-dimensional descending paper chromatography was used for the separation of amino acids and peptides. The most suitable combination of solvent systems was isopropanol-acetic acid-water (75:10:15 v/v/v) in machine direction and α-picoline-25% NH_4OH-water (70:2:28 v/v/v) in the other direction. Schleicher-Schüll 2043b Mgl paper was used.

The R_{Ala} values of various amino acids, amino sugars, and peptides are given in Table 2 and Table 3, respectively. The R_{Ala} values can vary slightly depending on the conditions used for chromatography (minor variation of solvent composition, temperature, etc.). Moreover, the R_{Ala} values obtained for peptides separated by one-dimensional chromatography are sometimes slightly different from that of two-dimensional chromatograms. The quantity of hydrolysate applied on the chromatograms is also important for the separation of the peptides and amino acids. The equivalent of 2 to 5 mg of cell walls was usually applied. It is advisable to standardize the system under definite chromatography conditions by using authentic amino acids, amino sugars, and peptides.

Some of the peptides form characteristic colors after spraying with ninhydrin and heating at 100 C. Peptides with N-terminal glycine residues appear yellow at the beginning and within 5 to 15 min turn to purple; the same is true for peptides with N-terminal threonine or serine residues. The latter are yellow or orange during the first 1 to 3 min after heating.

It is also possible to separate the peptides on the amino acid analyzer. Authentic peptides or isolated peptides from a partial acid hydrolysate of cell walls were applied to the amino acid analyzer to determine the exact position of the peptides. Figure 3 shows the separation of a partial hydrolysate of cell walls (4 N HCl, 100 C, 2 hr) of *Sarcina lutea* ATCC 383. The peptidoglycan of this strain contains a typical peptide subunit with L-Lys as diamino acid and the peptide subunits are cross-linked by

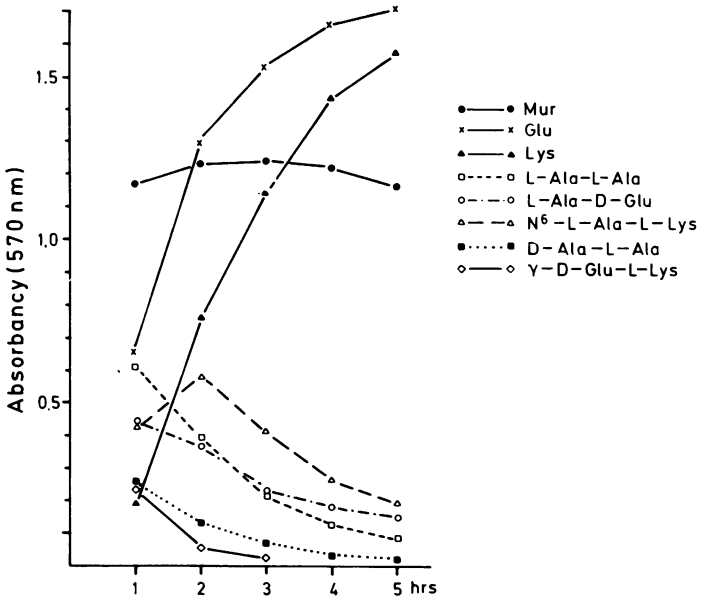

FIG. 2. *Quantitative release of various amino acids and peptides from cell walls of Sarcina lutea ATCC 383 during hydrolysis with 4 N HCl at 100 C as a function of time.*

TABLE 2. *R_{Ala} values of various amino acids and amino sugars after two-dimensional descending separation*[a]

Amino acid or amino sugar	R_{Ala} values	
	Isopropanol	α-Picoline
Alanine	1.0	1.0
Aspartic acid	0.55	0.28
Diaminobutyric acid	0.28	0.58
Diaminopimelic acid	0.11	0.14
Galactosamine	0.57	1.68
Glucosamine	0.60	1.80
Glutamic acid	0.81	0.29
Glycine	0.60	0.66
Homoserine	0.83	1.01
Threo-3-hydroxyglutamic acid	0.43	0.27
Lysine	0.30	0.42
Mannosamine	0.71	1.80
Muramic acid	1.01	1.70
Ornithine	0.27	0.41
Serine	0.59	0.81
Threonine	0.84	1.02

[a] Two-dimensional descending separation in solvent systems isopropanol (machine direction) and α-picoline on Schleicher-Schüll 2046b Mgl paper. Running time: each direction 2 × 24 hr. Temperature of chromatography chamber: 27 to 28 C.

tri-L-alanyl peptides. The dipeptides L-Ala-D-Glu, γ-D-Glu-L-Lys, and L-Lys-D-Ala are derived from the peptide subunit, whereas N^6-L-Ala-L-Lys, L-Ala-L-Ala, and D-Ala-L-Ala are typical for the interpeptide bridge and its connection to the peptide subunit.

Typical pictures of two-dimensional paper chromatograms of partial acid hydrolysates of cell walls are given in Fig. 4. The cell walls of these two organisms have almost identical amino acid composition, but the "finger prints" are quite different. This indicates that the amino acid sequences of the two peptidoglycans are unlike. The detailed analysis showed that the peptidoglycan of *Micrococcus luteus* belongs to subgroup A2 (*see below*), whereas that of *Bifidobacterium breve* belongs to subgroup A3 (vide infra).

If some peptides were not sufficiently well resolved in the two standard solvent systems (isopropanol and α-picoline), we used other systems, especially *n*-butanol-acetic acid-pyridine-water (420:21:280:210, v/v/v/v) and *n*-butanol-propionic acid-water (750:352:498 v/v/v) (344). For the separation of diaminopimelic acid (Dpm)-containing peptides, we applied the modified solvent system of Rhuland et al. (315), methanol-pyridine:formic acid:water (80:10:1:19 v/v/v/v), or high-voltage electrophoresis on Whatman no. 3MM paper (39).

The peptides were isolated by repeated one-dimensional paper chromatography (336) or by developing a two-dimensional chromatogram with ninhydrin and cutting out the corresponding areas from other unsprayed parallel two-dimensional chromatograms. In the case of

TABLE 3. *R_{Ala} values of various peptides from partial acid hydrolysates of cell walls after two-dimensional descending separation*[a]

Peptide	R_{Ala} values		Color[b]	Peptide	R_{Ala} values		Color[b]
	Isopropanol	α-Picoline			Isopropanol	α-Picoline	
D-Ala-L-Ala	1.17	1.20		L-Lys-D-Ala-L-Glu	0.37	0.27	
D-Ala-D-Ala or L-Ala-L-Ala	1.26	1.25		Mur-L-Ala	1.20	1.60	
L-Ala-L-Ala-L-Ala	1.30	1.32		Mur-Gly	0.98	1.39	
D-Ala-Gly	0.93	0.91		Mur-L-Ala-D-Glu	1.08	0.81	
D-Ala-Gly-Gly	0.78	0.83		Mur-GlcNH_2	0.30	1.36	
L-Ala-Thr	1.05	1.27					
L-Ala-D-Glu or D-Ala-L-Glu	0.95	0.23		N^2-Gly-L-Lys	0.27	0.53	Brownish
D-Ala-D-Asp	0.95	0.23		N^2-D-Ala-D-Lys	0.39	0.60	
D-Ala-D-Glu	1.05	0.29		N^6-Gly-L-Lys	0.22	0.62	Brownish
L-Ala-γ-D-Glu-L-Lys	0.19	0.32		N^6-L-Ser-L-Lys	0.24	0.77	Brownish
D-Ala-γ-L-Glu-Gly	0.67	0.31		N^6-L-Ala-L-Lys	0.40	0.87	
D-Ala-γ-L-Glu-L-Ala	0.92	0.41		N^6-Thr-L-Lys	0.32	0.91	
D-Ala-L-Ala-D-Glu	1.05	0.54		N^6-Gly-L-Lys-D-Ala	0.33	0.76	Yellow
D-Ala-Dpm	0.28	0.34		N^6-L-Ser-L-Lys-D-Ala	0.33	0.85	
				N^6-L-Ala-L-Lys-D-Ala	0.47	0.95	
				N^6-α-D-Glu-D-Lys	0.36	0.27	
D-Asp-L-Ala	0.55	0.33	Steel blue	N^6-γ-D-Glu-L-Lys	0.30	0.26	
Dpm-D-Ala	0.31	0.40		ε-(Aminosuccinyl-) lysine	0.32	0.77	
γ-D-Glu-L-Lys	0.20	0.23		N^6-(L-Ala-L-Ala)-L-Lys	0.46	1.0	
γ-L-Glu-Gly	0.56	0.27		N^6-(D-Ala-D-Asp)-L-Lys	0.39	0.97	
γ-L-Glu-L-Ala	0.92	0.40		N^6-(L-Ala-Thr)-L-Lys	0.40	1.02	
α-D-Glu-Gly	0.77	0.20		N^6-(D-Asp-L-Ala)-L-Lys	0.17	0.60	
D-Glu-Dpm-D-Ala	0.15	0.08					
Gly-Gly	0.61	0.59	Yellow	N^2-D-Ala-D-Orn	0.37	0.58	
Gly-Gly-Gly	0.51	0.62	Yellow	N^5-Gly-L-Orn	0.20	0.58	Brownish
Gly-Gly-Gly-Gly	0.39	0.64	Yellow	N^5-L-Ser-L-Orn	0.22	0.75	Brownish
Gly-L-Ser	0.50	0.74	Yellow	N^5-Gly, N^2-D-Ala-D-Orn	0.29	0.72	Yellow
Gly-L-Ala	0.90	0.85	Yellow	N^5-L-Ala-L-Orn	0.40	0.87	
Gly-D-Glu	0.72	0.17	Yellow	L-Orn-D-Ala	0.37	0.58	
Gly-Gly-L-Ala	0.71	0.83	Yellow				
Gly-α-Hyg-Gly	0.35	0.15	Yellow	L-Ser-L-Ala	0.93	1.01	Yellow
Gly-γ-Hyg-Hsr	0.36	0.20	Yellow	L-Ser-D-Glu	0.70	0.23	Yellow
Gly-L, L-Dpm-D-Ala	0.21	0.14	Brownish	L-Ser-Gly	0.66	0.80	Yellow
				L-Ser-L-Ser	0.55	0.90	Yellow
L-Lys-D-Ala	0.39	0.60					
L-Lys-D-Ala-D-Ala	0.52	0.87		Thr-L-Ala	1.16	1.36	Yellow

[a] Two-dimensional descending separation in solvent systems isopropanol (machine direction) and α-picoline on Schlescher-Schüll 2043b Mgl paper. Running time: each direction 2 × 24 hr. Temperature of chromatography chamber: 27 to 28 C.

[b] Most spots give the usual violet color with ninhydrin; only the unusual colors are specified.

Dpm-peptides preparative electrophoresis was applied (39). The isolated dipeptides were identified by determining the quantitative amino acid composition and the configuration of the amino acids and the N-terminal amino acid (336). The tri- and higher oligo-peptides were again subjected to partial acid hydrolysis, and the resulting smaller peptides were identified. The involvement of the β-carboxyl group of Asp or the γ-carboxyl group of Glu in a peptide bond was demonstrated by photolysis of the corresponding DNP-peptides (271, 288).

In many cases these four steps (quantitative amino acid composition, configuration of the amino acids, N- and C-terminal amino acids, and isolation and identification of oligopeptides after partial acid hydrolysis of cell walls) were sufficient to establish the amino acid sequence of the peptidoglycan.

Only in the case of very complicated structures were additional data necessary to decide which component belongs to the peptide subunit and which to the interpeptide bridge. To obtain this additional information, nucleotide

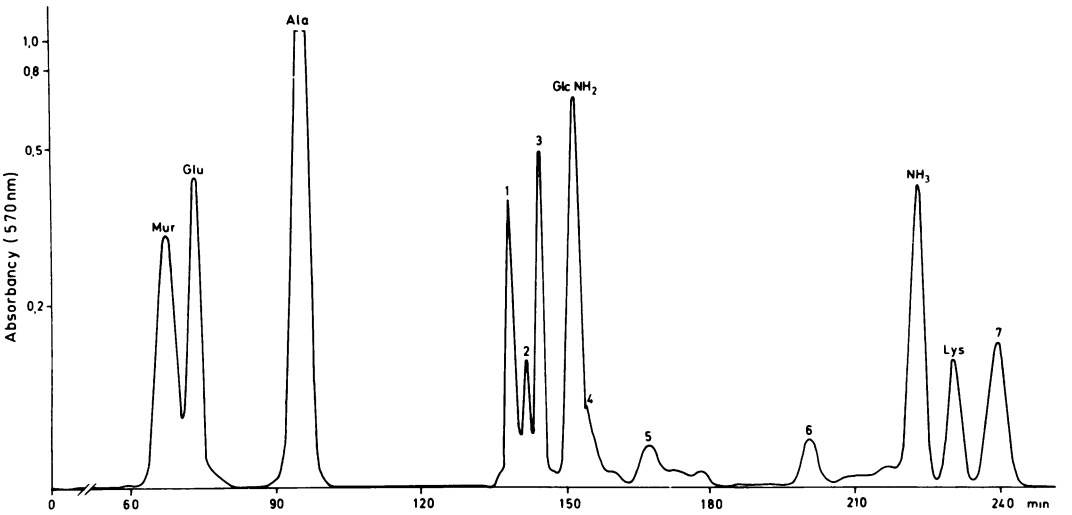

FIG. 3. *Elution profile of a partial acid hydrolysate (4 N HCl, 100 C, 2 hr) of cell walls of Sarcina lutea ATCC 383 separated on a model 120C Beckman amino acid analyzer. Conditions: one-column procedure. First, buffer A (0.2 M sodium citrate buffer, pH 3.24); after 100 min, buffer B (0.7 M sodium citrate buffer, pH 4.18). Temperature change from 30 to 55 C after 15 min. 1, L-Ala-D-Glu; 2, D-Ala-L-Ala; 3, L-Ala-L-Ala; 4, L-Lys-D-Ala; 5, γ-D-Glu-L-Lys; 6, unidentified peptide; 7, N^6-L-Ala-L-Lys.*

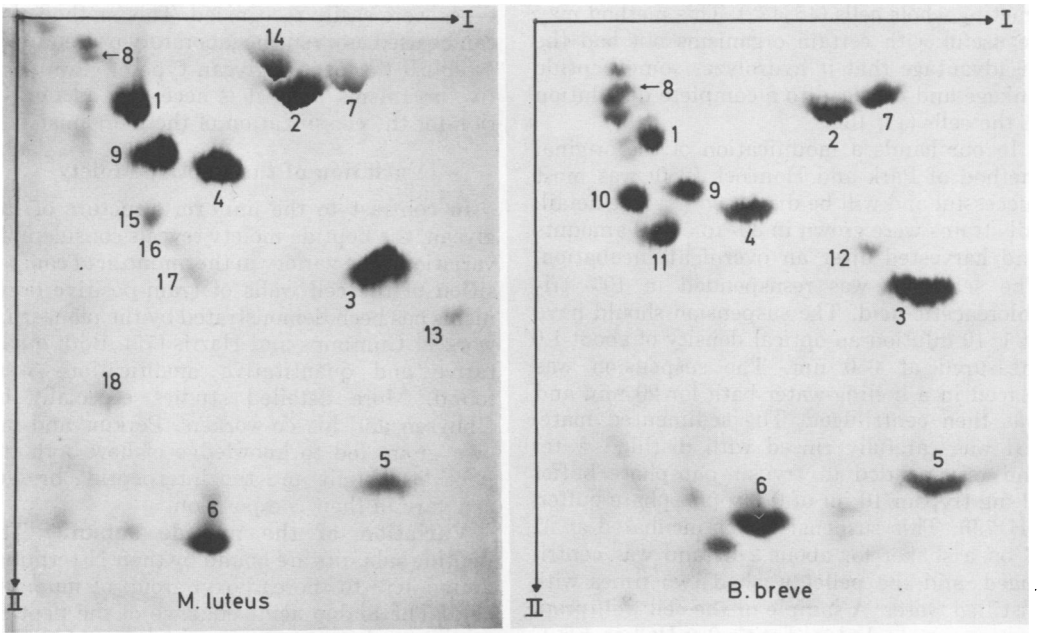

FIG. 4. *Two-dimensional chromatograms of partial acid hydrolysates (4 N HCl, 100 C, 1.5 hr) of cell walls of Micrococcus luteus (A2, Fig. 7) and Bifidobacterium breve (A3α, Fig. 23). I, isopropanol-acetic acid-water (75:10:15 v/v/v); II, α-picoline-25% NH_4OH-water (70:2:28 v/v/v). 1, Lys; 2, Glu; 3, Ala; 4, Gly; 5, Mur; 6, $GlcNH_2$; 7, L-Ala-D-Glu; 8, γ-D-Glu-L-Lys; 9, L-Lys-D-Ala; 10, N^6-Gly-L-Lys; 11, N^6-Gly-L-Lys-D-Ala; 12, D-Ala-Gly; 13, D-Ala-L-Ala; 14, D-Glu-Gly; 15, N^6-D-Ala-L-Lys; 16, N^6-D-Ala-L-Lys-D-Ala; 17, Lys-D-Ala-L-Ala; 18, Mur-$GlcNH_2$.*

precursors of the peptidoglycan, accumulating when growing bacteria were poisoned by vancomycin (336) or D-cycloserine (250, 341), were extracted. In some cases the precursors were isolated from noninhibited bacteria in the stationary-growth phase (81). The nucleotide precursors were extracted with cold trichloroacetic acid from the cells, and the neutralized extract

was subjected either to column chromatography on Dowex-1 (300) or to column chromatography on Sephadex G-25 (81, 323). Fractions absorbing at 260 nm and containing bound *N*-acetylamino sugar (313) were pooled and chromatographed one-dimensionally on Whatman no. 3MM in isobutyric acid-0.5 M ammonia (5:3 v/v) and afterwards in 1 M ammonium acetate-ethanol (5:2 v/v). The ultraviolet (UV)-absorbing and ninhydrin-positive bands were eluted, and the amino acid sequence was elucidated by applying the previously discussed methods. We also isolated muropeptides from lysozyme lysates of cell walls (403) and determined their amino acid sequence (346).

Rapid screening method. To use the peptidoglycan type as a criterion in the classification of gram-positive bacteria, it would be valuable to have a simple and rapid procedure. Especially the preparation of the cell walls is very time-consuming. Several extraction procedures were tried to prepare a relatively pure cell wall preparation from whole cells without disintegrating the cells by mechanical means.

Some authors have suggested NaOH for extracting whole cells (45, 172). This method may be useful with certain organisms but had the disadvantage that it hydrolyzes some peptide linkage and can lead to a complete dissolution of the cells (17, 18).

In our hands a modification of the original method of Park and Hancock (280) was most successful and will be discussed in some detail. All strains were grown in 30- to 50-ml amounts and harvested after an overnight incubation. The sediment was resuspended in 10% trichloroacetic acid. The suspension should have at 1:10 dilution an optical density of about 1.0 measured at 650 nm. The suspension was placed in a boiling-water bath for 20 min and was then centrifuged. The sedimented material was carefully rinsed with distilled water and resuspended in trypsin-phosphate buffer (2 mg trypsin/10 ml of 0.1 M phosphate buffer, *p*H 7.9). This suspension was incubated at 37 C on a shaker for about 2 hr and was centrifuged, and the pellet washed two times with distilled water. A sample of the cell wall preparation was hydrolyzed with 6 N HCl at 105 C for 6 hr to determine the quantitative amino acid content, and another sample was hydrolyzed with 4 N HCl in a boiling-water bath for 45 to 60 min. This partial acid hydrolysate was spotted on a two-dimensional paper chromatogram (Schleicher and Schüll 2043b, 29 by 30 cm) and was separated by descending chromatography in the solvent systems, isopropanol and α-picoline, in each direction overnight (about 14 hr). In some cases it was necessary to run each direction twice. The "finger prints" were compared with those of known peptidoglycan types and this comparison, together with the molar ratios of the amino acids, made it possible to recognize the peptidoglycan type.

The trichloroacetic acid-extracted, trypsin-treated cells yielded relatively clean cell wall preparations. In Table 4 the quantitative amino acid compositions of these preparations are compared with those of conventionally isolated and purified cell walls.

The cell wall preparations obtained by the short procedure contained minor contaminating amino acids. But the contamination was usually so low that it did not interfere with the determination of the peptidoglycan type. In particular the "finger prints" were not changed by these contaminating amino acids. In most cases it was sufficient to make only the finger prints to establish the peptidoglycan type.

This short procedure is very reliable and has been useful for the screening of a great number of organisms, whereby known peptidoglycan types were easily recognized. This method also can be used as a routine laboratory procedure to establish the peptidoglycan type of gram-positive organisms when it is necessary as a criterion for the classification of these organisms.

Variation of the Peptide Moiety

In contrast to the uniform structure of the glycan, the peptide moiety reveals considerable variation. The variety in the amino acid composition of the cell walls of gram-positive organisms has been demonstrated by the pioneering work of Cummins and Harris (75). Both qualitative and quantitative modifications were found. More detailed studies especially by Ghuysen and his co-workers, Perkins and our own group led to knowledge of how both the peptide subunit and the interpeptide bridges can vary in their composition.

Variation of the peptide subunit. The peptide subunits are bound by their N-terminal amino acid to the carboxyl group of muramic acid. The amino acid sequence of the peptide subunit and its variations are depicted in Fig. 5. The amino acid linked to muramic acid is usually L-Ala, but in some cases it can be replaced by Gly or L-Ser (129, 249, 250, 288, 289, 335, 341).

D-Glu in position 2 can be hydroxylated in a few coryneform bacteria and then threo-3-hydroxy-glutamic acid (3-Hyg) is found instead of D-Glu (342). The hydroxylation of Glu to 3-Hyg depends very much on the oxygen supply

TABLE 4. *Comparison of the quantitative amino acid composition of cell walls isolated by hot trichloroacetic acid treatment of whole cells (C-TCA) with that of cell walls isolated by mechanical disintegration of cells (CW-Tryp)*[a]

Organism	Prepn	Molar ratio of amino acids							
		Dpm	Lys	Glu	Ala	Gly	Ser	Leu	Ile
Staphylococcus aureus	C-TCA		1.1	1.0	1.9	4.5	0.20	0.08	0.04
	CW-Tryp		1.0	1.0	2.0	4.8	0.10		
Microbacterium flavum	C-TCA	0.7	0.1	1.0	1.6	0.2	0.10	0.20	0.08
	CW-Tryp	0.95		1.0	1.7				
Micrococcus luteus	C-TCA		0.85	1.0	1.85	1.1	0.11	0.15	0.06
	CW-Tryp		0.98	1.0	1.89	1.0			
Micrococcus mucilaginosus	C-TCA		0.85	1.0	2.50	0.08	0.20		
	CW-Tryp		0.95	1.0	2.50		0.20		

[a] All preparations were purified by digestion with trypsin. The content of non-peptidoglycan amino acids (leucine, isoleucine) is an indication of contamination of the preparation.

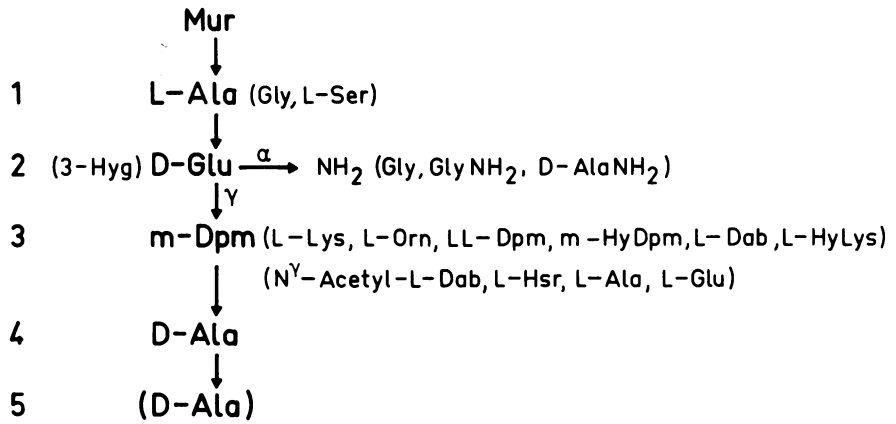

FIG. 5. *Variations of the peptide subunit. Amino acids in parentheses may replace the corresponding amino acids or substituents.*

during growth. Cells grown under microaerophilic conditions contain almost no 3-Hyg (343). The γ-carboxyl group of D-Glu or 3-Hyg is linked to the next amino acid in the peptide subunit. The α-carboxyl group is either free or substituted. In many organisms it is amidated (109, 389). In some bacteria like *Micrococcus luteus* it is substituted by Gly (252, 253, 338, 389). This Gly can be partly replaced by D-Ser when the organism is grown in a defined medium with a high content of D-Ser (419). In certain organisms the α-carboxyl groups of D-Glu are substituted by glycineamide as in *Arthrobacter atrocyaneus* (156) or by D-alanineamide as in *Arthrobacter* sp. NCIB 9423 (101).

The greatest variation occurs at position 3, where usually a diamino acid is found. The most widely distributed diamino acid is *meso*-diaminopimelic acid (m-Dpm). It is present in probably all gram-negative bacteria and in numerous other organisms, such as some species of bacilli, clostridia, lactobacilli, corynebacteria, propionibacteria, *Actinomycetales*, *Myxobacteriales*, *Rickettsiae*, and blue-green algae (427). Studies from different laboratories have shown that the L-asymmetric carbon of m-Dpm is bound in the peptide subunit. The γ-carboxyl group of D-Glu is linked to the amino group on the L-carbon of m-Dpm (50, 92, 93), and the amino group of D-Ala is linked to the carboxyl group on the same carbon of m-Dpm (394).

Since other amino acids known to be in position 3 are always L-isomers, it follows that the peptide subunit consists of amino acids with alternating L- and D-configuration. The carboxyl group of m-Dpm not engaged in a

peptide bond can be substituted by an amide group (188, 406).

L-Lysine is also a fairly common diamino acid at position 3. Less frequent are L-Orn (109, 167, 292, 301, 302, 427), L,L-Dpm (68, 69, 76, 345, 427), *meso*-2,6-diamino-3-hydroxy-β-pimelic acid (m-HyDpm) (290), and hydroxylysine (HyLys) (265, 356). Since all these amino acids possess an additional amino group, they are an excellent anchoring point for the cross-linking of the peptide subunits. Indeed, almost all peptide subunits containing one of these diamino acids in position 3 are cross-linked by means of these diamino acids.

In a few bacteria, however, the diamino acid in position 3 is not involved in the cross-linkage and remains unsubstituted (129, 343). In the case of *Corynebacterium insidiosum* the distant amino group of the diamino acid, L-diaminobutyric acid (L-Dab), is acetylated (289). In some coryneform bacteria the diamino acid in position 3 is replaced by a monoamino acid like L-homoserine (L-Hsr) (286, 291, 335), L-Ala or L-Glu (81). These types of peptide subunits are cross-linked in a different way. Since the amino acid in position 3 contains no reactive group for forming a peptide bond (*N*-acetyl-L-Dab, L-Hsr, L-Ala) or the group is unreactive (L-Lys, L-Orn, L-Dab, L-Glu), another trifunctional amino acid must be found as starting point of the cross-linking. The only other trifunctional amino acid besides the diamino acid occurring in the peptide subunit is D-Glu at position 2. Therefore, the cross-linking starts in these types at position 2.

Position 4 is almost always occupied by D-Ala, with very little variation. The carboxyl group of D-Ala is usually blocked by the interpeptide bridge, but a portion of the peptide subunits is not cross-linked (386). In such cases the C-terminal D-Ala is either split off if D-Ala carboxypeptidases are present (158), or it remains substituted by another D-Ala. Therefore, tri- and pentapeptides can also occur besides tetrapeptides, whereby the pentapeptide represents a remainder of the peptidoglycan precursor (369).

Variation of the mode of cross-linkage. Most variations of the peptide moiety of the peptidoglycan do not occur in the peptide subunit but in the interpeptide bridge and in the mode of cross-linkage. Ghuysen (109) divided the peptidoglycans into four different main types. Since then many new amino acid sequences of the peptidoglycan have been established and the knowledge of their biosynthesis is more complete. We shall use a new classification system based on the mode of cross-linkage and the proposed paths of biosynthesis. There are two main *groups* of cross-linkage called *A* and *B*, depending upon the anchoring point of the cross-linkage to the peptide subunit. They are divided in *subgroups* which carry *Arabic figures* and are characterized by the presence or absence of an interpeptide bridge, the kind of interpeptide bridges, and their mode of biosynthesis.

The *variations* within the subgroups reflect the diversity of the amino acids in position 3 of the peptide subunit. The variations are marked by *small Greek letters*. The variations can be subdivided into distinct peptidoglycan *types* based on the different amino acid sequence of the interpeptide bridges and on the differences in the substitution of the α-carboxyl group of D-glutamic acid. In the following chapter we shall discuss these different variations.

Besides a short descriptive text, figures and tables will be given for an easier understanding. The amino acid sequence of the various subgroups will be depicted in figures. The tables contain the kind of variation and all known types of each variation, together with the name of the organism in which the structure was first elucidated and the reference of the first description.

Group A: cross-linkage between position 3 and 4. The cross-linkage of group A extends from the ω-amino group of the diamino acid in position 3 of one peptide subunit to the carboxyl group of D-Ala in position 4 of another adjacent peptide subunit. This is the most common kind of cross-linkage. The first known example of this group is the directly cross-linked, m-Dpm containing peptidoglycan of *E. coli* (403) which we call variation A1γ.

A fragment of a subgroup A1 structure (direct cross-linkage) is depicted in Fig. 6. The amino group of the D-asymmetric carbon of m-Dpm forms a peptide bond with the carboxyl group of D-alanine of an adjacent peptide subunit. Since there is no interpeptide bridge involved, this kind of cross-linkage is called "direct

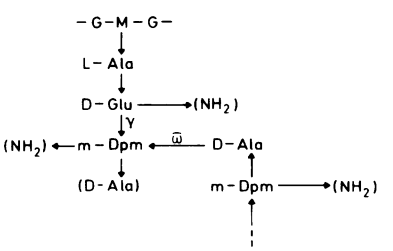

FIG. 6. *Fragment of the primary structure of a directly cross-linked, m-Dpm containing peptidoglycan (A1γ).*

cross-linkage." For many years it was only known to occur in the very widespread m-Dpm containing peptidoglycans. Recent investigations on the peptidoglycan structure of aerococci, several streptococci, and of *Spirochaeta* have shown that direct cross-linkage can also occur in bacteria containing L-Lys or L-Orn instead of m-Dpm. Thus, we can distinguish three variations within subgroup A1: variation A1α with L-Lys; variation A1β with L-Orn; variation A1γ with m-Dpm in position 3 of the peptide subunit (Table 5). The m-Dpm-containing variation may be subdivided in types depending on the amidation of the free carboxyl groups of the peptide subunits (Table 5). In *E. coli* and *Bacillus megaterium*, none of the carboxyl groups are amidated. In *B. licheniformis* ATCC 9945 the α-carboxyl group of D-Glu is substituted, whereas in *B. licheniformis* NCTC 6346 and in *B. subtilis* most of the peptide subunits have an amide substituent on the carboxyl group of the D-asymmetric carbon of m-Dpm. Cell walls of *Lactobacillus plantarum* and *Corynebacterium diphtheriae* have both carboxyl groups amidated. It should, however, be mentioned that the extent of amidation

TABLE 5. *Variations and types of subgroup A1*

Variation	Diamino acid	Amidation of peptide subunit	Species	Fig.	Reference
A1α	L-Lys	Partly amidated	*Aerococcus viridans, Gaffkya homari* ATCC 10400	18	268
A1β	L-Orn	Not amidated	*Spirochaeta stenostrepta*	28	Schleifer and Joseph, *manuscript in preparation*
A1γ	m-Dpm	Not amidated	*Escherichia coli, Bacillus megaterium*	6	394
		One carboxyl group amidated			
		(a) α-Carboxyl group of D-Glu	*Bacillus licheniformis* ATCC 9945	6	254
		(b) Carboxyl group of m-Dpm	*B. subtilis, B. licheniformis* NCTC 6346	6	148, 402
		Both carboxyl groups amidated	*Lactobacillus plantarum, Corynebacterium diphtheriae*	6	242, 406, 188

TABLE 6. *Peptidoglycan types of variation A3α*[a]

Position 4	Interpeptide bridge	Position 3	Species	Fig.	Reference
	→ Gly →		*Bifidobacterium*	23	176
	→ Gly_5 →		*Staphylococcus aureus* Copenhagen	8	113
	→ Gly(L-Ser) →		*Micrococcus mucilaginosus*	16	Schleifer et al., *manuscript in preparation*
	→ L-Ala(L-Ser) →	↑	*M. mucilaginosus*	16	Schleifer et al., *manuscript in preparation*
D-Ala →	→ L-Ala →	← L-Lys	*Arthrobacter crystallopoietes*	21c	210, 211
	→ $L\text{-}Ala_2$ →		*L. coprophilus*	21c	144
	→ $L\text{-}Ala_3$ →		*M. roseus*	13a	293
			Streptococcus thermophilus		336
	→ $L\text{-}Ala_4$ →		*A. ramosus*	13a	101
	→ $L\text{-}Ala_5$ →		*Micrococcus* sp. 7425		Schleifer, *unpublished data*

[a] Interpeptide bridges consist of single amino acids or homo-oligopeptides.

can be decreased by enzymic reactions (148a, 188).

Besides this variation in the amidation, there is also a variation in the length of the peptide subunit due to the variable occurrence of D-Ala carboxypeptidases. If these enzymes are present, peptide subunits consisting of tripeptides (lacking D-Ala) are found besides tetrapeptides (206, 242, 386, 402). In case of *L. plantarum* both carboxyl groups of m-Dpm in the tripeptides are amidated.

A fragment of the primary structure of subgroup A2 (cross-linkage by polymerized peptide subunits) is depicted in Fig. 7. This subgroup shows several peculiarities. (i) At least 50% of the *N*-acetylmuramic residues are not substituted by a peptide subunit (220, 265). (ii) All of the glycine residues in the native cell walls are C-terminal (388, 389). Therefore, Gly is not involved in the cross-linking of the peptide subunits but is bound to the α-carboxyl group of D-Glu (189, 253). (iii) Eighty percent of the ϵ-amino groups of L-Lys are unsubstituted. The primary structure of the peptidoglycan was first established by studies in our own laboratory (338) and later confirmed and refined by the work of Ghuysen's group (116).

According to these findings, the peptide subunits are connected through bridges which are formed by a "head-to-tail" linkage of several peptide subunits. Thus, the peptide subunits are linked together through polymerized peptide subunits. Up to four peptide subunits can form the connecting bridge.

Subgroup A2 may be understood as a further development of the directly cross-linked variation of A1α. Besides the substitution of the α-carboxyl group of D-Glu by Gly, the only difference from the directly cross-linked variation is an additional biosynthetic step as proposed by Schleifer and Kandler (338): that is an amidase reaction in which the amide linkage between *N*-acetylmuramic acid and L-Ala is hydrolyzed after the peptide subunit has undergone a direct cross-linkage between the C-terminal D-Ala and the ϵ-amino group of L-Lys of an adjacent peptide subunit. The head-to-tail linkage is then formed by a second transpeptidation reaction. But this time the transpeptidation results between the D-Ala and the N-terminal L-Ala. When this process is repeated, interpeptide bridges consisting of several "polymerized" peptide subunits can be formed. It may be possible that for the two transpeptidations two different transpeptidases are necessary, depending on the structure of the amino acceptor: ϵ-amino group of L-Lys or α-amino group of L-Ala (116).

Up to now there are no known variations of this subgroup. A proposal that the L,L-Dpm-containing peptidoglycan of *Clostridium perfringens* possesses a similar structure (296) could not be confirmed (221, 345).

Subgroup A3 (cross-linkage by interpeptide bridges consisting of monocarboxylic L-amino acids or glycine, or both) is very common among gram-positive bacteria. Actually, the first known primary structure of the peptidoglycan of a gram-positive bacterium, the peptidoglycan of *Staphylococcus aureus* strain Copenhagen (113) belongs to this subgroup. A pentaglycine chain serves as interpeptide bridge in this case. A typical fragment of such a peptidoglycan is illustrated in Fig. 8. There exist several variations of subgroup A3, depending upon the diamino acid occurring in position 3 of the peptide subunit.

The most common variation is A3α. In this case L-Lys is found in position 3. The known peptidoglycan types of this variation are summarized in Tables 6 and 7. Table 6 comprises the interpeptide bridges consisting of a single amino acid residue or of homo-oligopeptides. The size of the interpeptide bridge varies between one and six amino acids. The homo-oligopeptides are composed of Gly or L-Ala

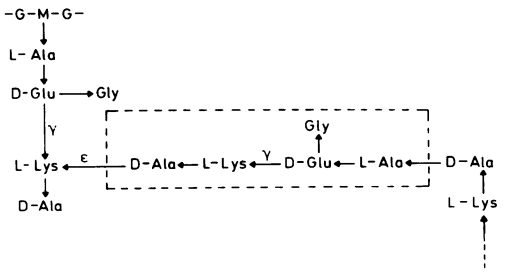

FIG. 7. *Fragment of the primary structure of a peptidoglycan of subgroup A2 (Micrococcus luteus). The cross-linkage bridge is marked by a dashed frame.*

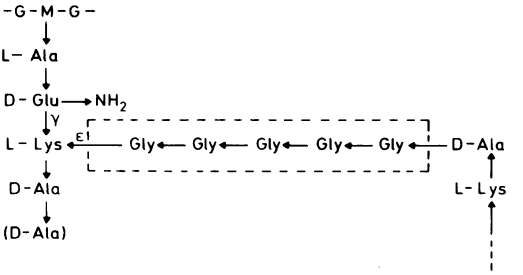

FIG. 8. *Fragment of the primary structure of a peptidoglycan of subgroup A3 (Staphylococcus aureus, strain Copenhagen). Interpeptide bridge marked by a dashed frame.*

TABLE 7. *Peptidoglycan types of variation A3α*[a]

Position 4	Interpeptide bridge	Position 3	Species	Fig.	Reference
D-Ala →	→ Gly → L-Ala →	ε→ L-Lys	*Peptostreptococcus* sp.	19d	339
	→ Gly_{4-5} → L-Ala →		*Staphylococcus epidermidis*	12b	334
	→ Gly_{2-4} → $L\text{-}Ser_{1-2}$ → Gly →		*S. epidermidis*	12c	346, 385
	→ L-Ala → Gly_{5-6} →		*S. roseus*	14e	Schleifer et al., *manuscript in preparation*
	→ L-Ala → Gly → $L\text{-}Ala_2$ →		*Streptococcus* sp.	19e	Hladny 1972[b]
	→ L-Ala(L-Ser) → $L\text{-}Ala_2$ →		*Streptococcus* group G	19c	Hladny 1972
	→ L-Ala(L-Ser) → L-Ala →		*S. agalactiae*	19b	Hladny 1972
	→ L-Ser → L-Ala →		*Lactobacillus viridescens*	21a	174
	→ L-Ser → L-Ala(L-Ser) →		*Leuconostoc gracile*	21a	169
	→ $L\text{-}Ala_a$ → L-Ser →		*L. cremoris*	21b	174
	→ Gly → L-Thr →		*Streptococcus salivarius*	20b	141a
	→ L-Ser(L-Ala) → L-Thr →		*S. bovis*	20a	141a
			S. equinus		
	→ L-Ala → L-Thr →		*Streptococcus* sp.	25a	337
	→ $L\text{-}Ala_2$ → L-Thr →		*Arthrobacter citreus*	26a	Fiedler et al. 1972[c]
	→ $L\text{-}Ala_3$ → L-Thr →		*Micrococcus roseus* R 27	13b	293
	→ $L\text{-}Ala_2$ → L-Ser → L-Thr →		*Mycococcus ruber*		Schleifer, *unpublished data*
	→ L-Ala → L-Thr → L-Ala →		*A. aurescens*	26b	Fiedler et al. 1972[c]
	→ L-Ala → L-Thr → L-Ser →		*A. polychromogenes*	26c	Fiedler et al. 1972[c]

[a] Interpeptide bridges consist of hetero-oligo peptides.
[b] J. Hladny, Ph.D. thesis, Technical University, Munich, 1971.
[c] F. Fiedler, K. H. Schleifer, and O. Kandler, J. Bacteriol. **113:** 8–17.

residues. In case of *M. mucilaginosus*, L-Ser partially replaces Gly or Ala.

Table 7 shows interpeptide bridges consisting of hetero-oligopeptides. The size of the interpeptide bridges ranges from two to seven amino acid residues. Especially interesting are interpeptide bridges with the same amino acid composition but a different sequence. For example, in the case of the peptidoglycan of *Arthrobacter polychromogenes*, L-Ser is bound to the ε-amino group of L-Lys and L-Thr is bound to L-Ser; whereas, in the case of *Micrococcus ruber*, the sequence is reversed. This indicates that it is important to determine the sequence, since even the quantitative amino acid composition is not sufficient to distinguish the different peptidoglycans. The problem of the biosynthetic fixation of the amino acid sequence of an interpeptide bridge has not yet been solved. The biosynthesis of an interpeptide bridge consisting of a hetero-oligopeptide has been studied in greater detail only in the case of *L. viridescens* (305). In vitro studies have revealed that L-Ser is attached with about the same frequency as L-Ala to the ε-amino group of L-Lys, whereas in cell walls it is almost exclusively L-Ala that is linked to this group. Variation A3β and variation A3γ are far less frequent than variation A3α. Variation A3β contains L-Orn in position 3 of the peptide subunit and has been found up to now in three different peptidoglycan types (Table 8).

The distinction between variation A3α and A3β is sometimes not possible since both diamino acids (L-Lys, L-Orn) can be present in varying amounts in the same peptidoglycan (145). It has been shown in our laboratory that the ratio of L-Lys/L-Orn may be different from strain to strain, but is not significantly influenced by the amount of lysine or ornithine in the medium.

Variation A3γ reveals L,L-Dpm in position 3 of the peptide subunit. Only two peptidoglycan types of this variation have been found so far (Table 8).

Subgroup A4 (cross-linkage by interpeptide bridges containing a dicarboxylic amino acid) was discussed by Ghuysen (109) together with subgroup A3 as "peptidoglycans of type II." At the time Ghuysen wrote his review, only one peptidoglycan type of subgroup A4 was known, but meanwhile different variations and various

TABLE 8. *Peptidoglycan types of variations A3β and A3γ*

Variation	Position 4	Interpeptide bridge	Position 3	Species	Fig.	Reference
		→ Gly_{2-3} →		*Micrococcus radiodurans*	14f	109
A3β	D-Ala →	→ $L\text{-}Ala_3$ →	ε, L-Orn	*B. globosum*	22c	145
		→ L-Ala → L-Thr → L-Ala → L-Ser → → Gly →		*B. longum*	22b	200
				Propionibacterium petersonii	15a	345
A3γ	D-Ala →		ω, L, L-Dpm	*S. roseochromogenes*	15a	19a
		→ Gly_3 →		*Arthrobacter tumescens*	27a	101

peptidoglycan types of this subgroup have been established. We feel that the differentiation between subgroup A3 and subgroup A4 is justified by the different modes of biosynthesis of their interpeptide bridges. The L-amino acids and glycine of the interpeptide bridge of subgroup A3 are activated as their transfer ribonucleic acid (tRNA) derivatives and added sequentially to the ω-amino group of the L-diamino acid (240). In the case of subgroup A4, however, the dicarboxylic amino acids are always linked in the interpeptide bridge by their distant (β or γ) carboxyl group, and the incorporation takes place without the participation of a tRNA derivative. For D-aspartic acid, it was shown that it is activated as D-aspartyl-β-phosphate and is incorporated into a lipid intermediate of the peptidoglycan of *Streptococcus faecium* and *Lactobacillus casei* (362). The γ-linked L- or D- Glu residues may be synthesized via their γ-phosphate derivaties in analogy to the D-aspartyl residues.

The α-carboxyl group of the dicarboxylic amino acid is free or it is substituted by an amide. A typical fragment of the primary structure of a peptidoglycan of subgroup A4 is depicted in Fig. 9. As an example, the peptidoglycan of *S. faecium* was chosen. As in the case of subgroup A3, one also finds three variations in subgroup A4 depending on the diamino acid occuring in position 3 of the peptide subunit. The most common variation is A4α with L-Lys in position 3. The various known peptidoglycan types of this variation are summarized in Table 9. The peptidoglycan types of the less common variations A4β and A4γ, containing L-Orn or m-Dpm, are listed in Table 10.

Group B: cross-linkage between positions 2 and 4. Peptidoglycan group B (cross-linkage between positions 2 and 4) is much less frequent than group A. It is found only among some coryneform bacteria, especially the plant pathogenic corynebacteria. The cross-linkage in group B extends from the α-carboxyl group of D-Glu of one peptide subunit to the carboxyl group of D-Ala of an adjacent peptide subunit. Since the cross-linkage occurs between two carboxyl groups, a diamino acid has to be present in the interpeptide bridge. This group also shows the greatest variation in the composition of the peptide subunit. (i) The N-terminal amino acid of the peptide subunit which is bound to the carboxyl group of muramic acid is not L-Ala as in group A but Gly or L-Ser. (ii) The D-glutamic acid in position 2 is sometimes hydroxylated and threo-3-hydroxyglutamic acid is found instead of D-Glu. (iii). The diamino acid at position 3, if it is present, is not involved in the cross-linkage and reveals an unsubstituted ω-amino group (in the case of L-Lys and L-Orn) or the ω-amino group is acetylated as in some cases of L-Dab. In most cases, however, the "unnecessary" diamino acid in the peptide subunit is replaced by a monoamino acid such as L-Hsr, L-Ala, or L-Glu. Typical fragments of the primary structures of peptidoglycans of group B are depicted in Fig. 10 and 11. Group

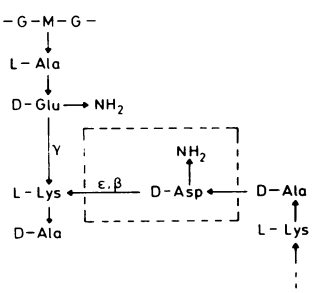

FIG. 9. *Fragment of the primary structure of a peptidoglycan of subgroup A4 (Streptococcus faecium, many lactobacilli). Interpeptide bridge marked by a dashed frame.*

TABLE 9. *Peptidoglycan types of variation A4α*

Position 4	Interpeptide bridge	Position 3	Species	Fig.	Reference
	→ D-Asp $\xrightarrow{\beta}$ (↓ NH_2)		*Streptococcus lactis*	9	337
			S. faecium	9	115
	→ D-Asp $\xrightarrow{\beta}$ L-Ala →		*Bacillus pasteurii*	24b	H. Ranftl, and O. Kandler, *manuscript in preparation*
	→ Asp $\xrightarrow{\beta}$ L-Ser →		*C. manihot*		101
D-Ala →	→ D-Glu $\xrightarrow{\gamma}$	$\xrightarrow{\epsilon}$ L-Lys	*Planococcus* sp.	17a	340
	→ D-Glu $\xrightarrow{\gamma}$ Gly →		*Sporosarcina ureae*	17b	340
	→ L-Glu $\xrightarrow{\gamma}$ Gly →		*Micrococcus luteus* ATCC 398	14a	271
	→ L-Glu $\xrightarrow{\gamma}$ L-Ala →		*M. freudenreichii* ATCC407	14b	271
	→ D-Glu $\xrightarrow{\gamma}$ L-Ser →		*M. cyaneus* CCM 856	14d	Schleifer et al., *manuscript in preparation*
	→ D-Glu $\xrightarrow{\gamma}$ $L\text{-Ser}_2$ →		*M. nishinomiyaensis* CCM 2140	14d	Schleifer et al., *manuscript in preparation*

TABLE 10. *Peptidoglycan types of variations A4β and A4γ*

Variation	Position 4	Interpeptide bridge	Position 3	Species	Fig.	Reference
		→ D-Asp $\xrightarrow{\beta}$ (↓ NH_2)		*Lactobacillus cellobiosus*	9	302, 303
		→ D-Asp $\xrightarrow{\beta}$ D-Ser →		*Bifidobacterium bifidum*	22a	199[a], 395
A4β	D-Ala →	D-Asp $\xrightarrow{\beta}$ (↓)	$\xrightarrow{\delta}$ L-Orn (↓)	*Cellulomonas flavigena*		Fiedler and Kandler, *manuscript in preparation*
		→ D-Glu →		*C. fimi*		Fiedler and Kandler, *manuscript in preparation*
		→ D-Glu $\xrightarrow{\gamma}$ (↓ NH_2)		*C. biazotea*		Fiedler and Kandler, *manuscript in preparation*
		→ Asp $\xrightarrow{\beta}$		*Arthrobacter duodecadis*	27b	Bogdanovaky et al., *manuscript in preparation*
A4γ	D-Ala →		$\xrightarrow{\omega}$ m-Dpm			
		→ D-Glu $\xrightarrow{\gamma}$ D-Glu $\xrightarrow{\gamma}$		*Micrococcus conglomeratus*	15b	39

[a] Koch et al. (199) reported L-Ser, whereas Veerkamp (395) found D-Ser. A reinvestigation of the cell wall prepared by Koch et al. (199) in our laboratory confirmed the occurrence of D-Ser.

B can be subdivided into two subgroups due to the occurrence of L- or D- diamino acids in the interpeptide bridges.

Subgroup B1 (interpeptide bridge containing an L-diamino acid) includes all peptidoglycan types containing L-diamino acids in the interpeptide bridge which are linked by their ω-amino group to the carboxyl group of a D-Ala. It can be divided into four variations, depending upon the amino acid found in position 3 of the peptide subunit. The different variations are listed in Table 11. In variation B1α, L-Eys occurs in position 3; in B1β, L-Hsr; in B1γ, L-Glu; and in B1δ L-Ala. The interpeptide bridge consists of a diamino acid residue which is L-Lys in all cases known so far, and, in addition, one or two glycine residues. Subgroup B2 (interpeptide bridge containing a D-diamino acid) covers all peptidoglycan types containing, in the interpeptide bridge, a D-diamino acid which is linked with its α-amino group to the carboxyl group of D-Ala in position 4. The primary structure of this subgroup is depicted in Fig. 11. As in the case of subgroup B1, subgroup B2 can also be divided into several variations, depending upon the amino acid occurring in position 3 of the peptide subunit. The known variations and peptidoglycan types are summarized in Table 12.

In variation B2α, L-Orn occurs in position 3; in B2β, L-Hsr; and in B2γ, L-Dab. Three different D-diamino acids were found in the interpeptide bridge. The α-linkage between D-Lys or D-Orn and D-Ala was established in different laboratories and by different methods (129, 249, 291, 335). The linkage of D-Dab to D-Ala had not been elucidated by the studies of Perkins (289, 291), but recent studies in our

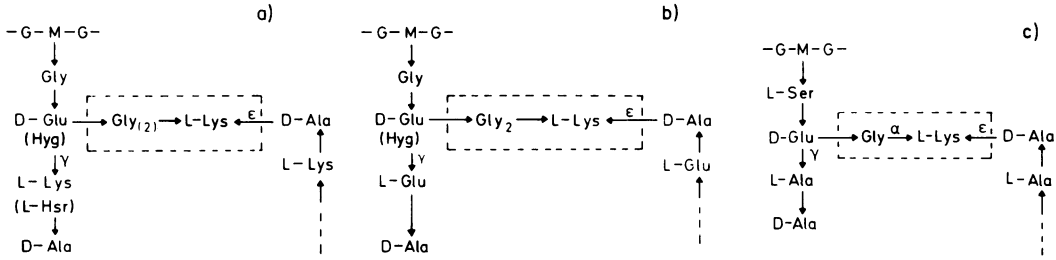

FIG. 10. *Fragments of primary structures of peptidoglycans of subgroup B1. (a) B1α (Microbacterium lacticum) and B1β (in Brevibacterium imperiale, the amino acids in parentheses are found); (b) B1γ (Arthrobacter sp. strain J39); (c) B1δ (Erysipelothrix rhusiopathiae).*

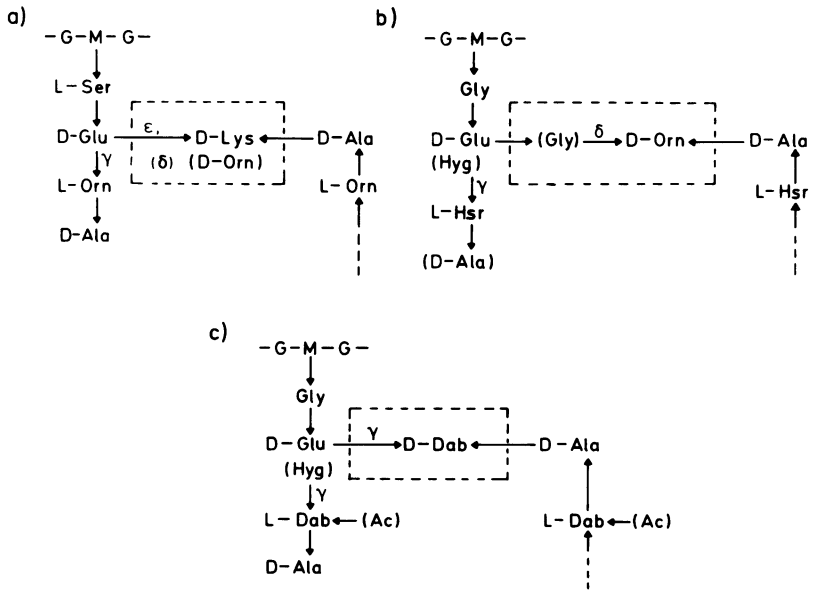

FIG. 11. *Fragments of the primary structures of peptidoglycans of subgroup B2. (a) (Butyribacterium rettgeri); (b) B2β (Corynebacterium poinsettiae); (c) B2γ (C. insidiosum, C. michiganensis).*

laboratory have indicated that the α-amino group of D-Dab is involved in forming the peptide bond with the carboxyl group of D-Ala of an adjacent peptide subunit.

Summary of the New Classification System of Peptidoglycans

Group A: cross-linkage between positions 3 and 4 of two peptide subunits.
- Subgroup A1: direct cross-linkage
  - Variation A1α: L-Lys in position 3 (1 type, Fig. 18)
  - Variation A1β: L-Orn in position 3 (1 type, Fig. 28)
  - Variation A1γ: m-Dpm in position 3 (3 types, Fig. 6, Table 5)
- Subgroup A2: cross-linkage by polymerized peptide subunits (1 type, Fig. 7)
- Subgroup A3: cross-linkage by interpeptide bridges consisting of monocarboxylic L-amino acids or glycine, or both.
  - Variation A3α: L-Lys in position 3 (23 types, Tables 6 and 7)
  - Variation A3β: L-Orn in position 3 (3 types, Table 8)
  - Variation A3γ: L,L-Dpm in position 3 (2 types, Table 8)
- Subgroup A4: cross-linkage by interpeptide bridges containing a dicarboxylic amino acid.
  - Variation A4α: L-Lys in position 3 (9 types, Table 9)
  - Variation A4β: L-Orn in position 3 (5 types, Table 10)
  - Variation A4γ: m-Dpm in position 3 (2 types, Table 10)

Group B: cross-linkage between positions 2 and 4 of two peptide subunits.
- Subgroup B1: interpeptide bridge containing an L-diamino acid.
  - Variation B1α: L-Lys in position 3 (1 type, Fig. 11, Table 11)
  - Variation B1β: L-Hsr in position 3 (1 type, Fig. 11, Table 11)

TABLE 11. *Peptidoglycan types of subgroup B1*

Variation	Position 3	Position 2	Interpeptide bridge	Position 4	Species	Fig.	Reference
B1α	L-Lys	$\xleftarrow{\gamma}$ D-Glu (Hyg)	$\rightarrow$ Gly $\xrightarrow{\alpha}$ L-Lys $\xleftarrow{\epsilon}$	D-Ala	*Microbacterium lacticum*	10a	343
B1β	L-Hsr	$\xleftarrow{\gamma}$ D-Glu (Hyg)	$\rightarrow$ Gly_2 $\xrightarrow{\alpha}$ L-Lys $\xleftarrow{\epsilon}$	D-Ala	*Brevibacterium imperiale*	10a	335
B1γ	L-Glu	$\xleftarrow{\gamma}$ D-Glu (Hyg)	$\rightarrow$ Gly_2 $\xrightarrow{\alpha}$ L-Lys $\xleftarrow{\epsilon}$	D-Ala	*Arthrobacter* J. 39	10b	81
B1δ	L-Ala	$\xleftarrow{\gamma}$ D-Glu	$\rightarrow$ Gly $\xrightarrow{\alpha}$ L-Lys $\xleftarrow{\epsilon}$	D-Ala	*Erysipelothrix rhusiopathiae*	10c	Fiedler and Kandler, *manuscript in preparation*

TABLE 12. *Peptidoglycan types of subgroup B2*

Variation	Position 3	Position 2	Interpeptide bridge	Position 4	Species	Fig.	Reference
B2α	L-Orn	$\xleftarrow{\gamma}$ D-Glu	$\xrightarrow[\delta]{\epsilon}$ D-Lys $\xleftarrow{\alpha}$ (D-Orn)	D-Ala	*Butyribacterium rettgeri*	11a	130
B2β	L-Hsr	$\xleftarrow{\gamma}$ D-Glu	$\xrightarrow{\delta}$ D-Orn $\xleftarrow{\alpha}$	D-Ala	*Corynebacterium poinsettiae*	11b	288
	L-Hsr	$\xleftarrow{\gamma}$ D-Glu (Hyg)	$\rightarrow$ Gly $\xrightarrow{\delta}$ D-Orn $\xleftarrow{\alpha}$	D-Ala	*Microbacterium liquifaciens*	11b	335
B2γ	L-Dab	$\xleftarrow{\gamma}$ D-Glu	$\rightarrow$ D-Dab $\xleftarrow{\alpha}$	D-Ala	*C. insidiosum* *C. michiganensis*	11c	291 Hammes et al., *manuscript in preparation*

Variation B1γ: L-Glu in position 3 (1 type, Fig. 11, Table 11)
Variation B1δ: L-Ala in position 3 (1 type, Fig. 11, Table 11)
Subgroup B2: interpeptide bridge containing a D-diamino acid.
Variation B2α: L-Orn in position 3 (1 type, Fig. 12, Table 12)
Variation B2β: L-Hsr in position 3 (2 types, Fig. 12, Table 12)
Variation B2γ: L-Dab in position 3 (1 type, Fig. 12, Table 12)

Stability of Peptidoglycan Structure Under Different Conditions of Growth

To establish the value of the peptidoglycan structure as a taxonomic criterion, it is necessary to demonstrate its phenotypic stability and to determine whether structural changes dependent on the growth phase or environmental factors can be observed. Changes of the cell wall composition have been observed only under conditions where different nutrients are limiting factors for growth (98, 99, 378) or in the presence of a quite unbalanced growth medium. Since detailed studies about these subjects will be published elsewhere (99a; Schliefer and Kandler, *manuscript in preparation*), we can restrict ourselves to a short survey of the phenotypic variations of the peptidoglycan.

Modifications of the peptidoglycan are not as dramatic as those of the cell wall polysaccharides (98, 99a, 378); in particular the type of peptidoglycan is rather stable, and, up to now, no drastic alterations of the peptidoglycan types have been found.

A few rather similar amino acids may be exchanged. In the case of *M. luteus* (*lysodeikticus*), glycine bound to the α-carboxyl group of glutamic acid can be replaced by D-serine (128, 419). In strains of staphylococci, glycine residues of the interpeptide bridges can be replaced by L-serine (52, 334, 346, 433) or L-alanine (334, 346). But these alterations only occur when these amino acids are added in rather high concentrations to the growth medium and when the original amino acids are present in limiting amounts. The same is true for the incorporation of hydroxylysine instead of lysine into the peptidoglycans of *S. faecalis* and *Leuconostoc* (346, 359) or for that of lanthionine, a monosulfur analogue of Dpm, instead of Dpm in a Dpm-auxotroph mutant of *E. coli* (198). The most pronounced phenotypic variation of the peptidoglycan was found among staphylococci (334, 346; G. Rauch, Diplom thesis, University of Munich, 1970; W. Hammes, Diplom thesis, University of Munich, 1970). These studies have indicated that the peptidoglycan composition is fixed genetically but that phenotypic alterations are possible. Under extreme conditions, two genetically different strains can even appear to be phenotypically similar. For example, the peptidoglycan of *S. aureus* normally contains little or no L-Ser, whereas that of *S. epidermidis* has a rather high serine content. When *S. aureus* is grown in a serine-enriched medium, however, its peptidoglycan now reveals a similar chemical composition to that of a *S. epidermidis* grown on a serine deficient medium. Despite the phenotypic resemblance of these two peptidoglycans under unusual conditions, the genetic differences are obvious if these organisms are cultivated in identical balanced media. The phenotypic variations of the staphylococcal peptidoglycans are rather an exception than a rule for the modifiability of peptidoglycans. Studies in our laboratory and other laboratories (373, 430) have indicated that the amino acid composition of the cell walls of various organisms grown in several different media did not vary significantly.

Some organisms normally contain two amino acids alternately at the same position, e.g., strains of *Bifidobacterium adolescentis* contain both L-Lys and L-Orn, in varying amounts in the same peptidoglycan. Hereby the ratio L-Lys/L-Orn varies with different strains but is not dependent upon the amount of Lys or Orn in the growth medium. A similar constancy was found among strains of *Leuconostoc gracile* which contain interpeptide bridges alternately formed by L-Ser-L-Ala and L-Ser-L-Ser (Lauer and Kandler, *manuscript in preparation*). The variation of the serine content is not caused by the amino acid composition of the growth medium since the serine content of the cell wall changed only very little when the strains were grown in a serine-enriched medium. These findings indicate that in both organisms, *B. adolescentis* and *L. gracile*, differences in the ratio of the amino acids in question are genetic properties.

In conclusion one can say that the only significant phenotypic alterations of amino acid composition in the peptidoglycan from cells without growth limitations were found among staphylococci. But even these alterations are not so extensive that the peptidoglycan type is considerably changed. In other words, a glycine-rich staphylococcal peptidoglycan will never be converted to an alanine-rich micrococcal peptidoglycan. The only possible modification of the peptidoglycan is seen in organisms grown in a serine-enriched and glycine-limited

medium which can alter their Lys-Gly_5 type to a Lys-Gly_4, L-Ser type. To avoid such a complication, the staphylococci should always be cultivated in a balanced medium, sufficient in glycine. By growing the bacteria in batch cultures under balanced conditions, there will be no phenotypic alterations of the peptidoglycan types.

SUMMARY

A comparison of the different amino acid sequence of peptidoglycans reveals the following rules for the chemical construction of the peptide moiety.

(i) The number of amino acids occurring in the various peptidoglycans is rather restricted. Between three and six chemically different amino acids can be found in a particular peptidoglycan. Branched amino acids (valine, leucine, etc.), aromatic amino acids (phenyl-alanine, tyrosine, tryptophan), sulfur-containing amino acids (cysteine, methionine), histidine, arginine, and proline have never been detected in clean preparations of peptidoglycans.

(ii) The tetrapeptide subunits show an alternating sequence of L- and D-amino acids. Even m-Dpm follows the rule since it is bound with its L-asymmetric center in the peptide subunit. D,D-Dpm residues found in the peptidoglycan of *B. megaterium* probably do not break the rule either. In vitro studies by Strominger and co-workers (370, 421) have shown that D,D-Dpm can replace the C-terminal D-Ala residue of a peptide subunit by transpeptidation. Therefore, it was suggested (370) that the occurrence of D,D-Dpm in the peptidoglycan of *B. megaterium* could be accounted for by these transpeptidation reactions. The only exception is the replacement of L-alanine in position 1 by glycine, but the latter amino acid does not have an asymmetric carbon atom. The pentapeptide subunit of the peptidoglycan precursor contains a D-D sequence (D-Ala-D-Ala) at position 4 and 5. These pentapeptides can be preserved as a minor portion of the peptide subunits in peptidoglycans lacking D,D-carboxypeptidase.

(ii) D-Glutamic acid in position 2 and D-alanine in position 4 and 5 of the peptide subunit are not usually replaced by other amino acids. In the case of position 2, however, a hydroxylation of D-glutamic acid to threo-3-hydroxyglutamic acid is possible in the peptidoglycans of a few coryneform bacteria. The D-alanine residues in position 4 and 5 can be replaced by other D-amino acids or glycine when the latter amino acids are present at rather high concentrations in the growth medium. It has been shown in our laboratory that under these extreme conditions the degree of cross-linkage of the peptide subunits is lower and the growth of the organisms is inhibited. Position 1 and position 3 of the peptide subunit can be occupied by different amino acids in diverse peptidoglycans. L-Alanine, L-serine, or glycine are alternates in position 1. Position 3 shows the greatest diversity. Nine different amino acids have been found in this position (Fig. 5).

(iv) Most of the amino acids found in the peptide subunits can also be used in the construction of the interpeptide bridges. The monocarboxylic, monoamino acids of the interpeptide bridges usually occur as L isomers. An exception is D-serine found in the interpeptide bridge of the peptidoglycan of *Bifidobacterium bifidum*. Dicarboxylic amino acids and diamino acids, on the other hand, can occur in both configurations in the interpeptide bridges. There is probably also a different mode of biosynthesis for these sterically different kinds of interpeptide bridges.

The monocarboxylic L-amino acids and glycine are activated as amino acyl-tRNA for incorporation into the interpeptide bridges (58, 294, 316, 317). This is not possible for the dicarboxylic amino acids, since these amino acids are linked by their distant carboxyl groups (β or γ) in the interpeptide bridges and not by their α-carboxyl group as is usual in proteins. These unusual peptide bonds are presumably synthesized via their corresponding phosphate derivatives (aspartyl-β-phosphate or glutamyl-γ-phosphate). The activation of D-aspartic acid via D-aspartyl-β-phosphate was proved in vitro in the case of enzyme preparations obtained from *S. faecalis* and *L. casei* (362).

A similar kind of activation may be proposed in the case of D-serine. The mode of biosynthesis of the interpeptide bridges which contain diamino acids has not yet been unraveled. The participation of tRNA can be excluded since the carboxyl groups of these diamino acids do not take part in the formation of the interpeptide bridge. It can be assumed that these diamino acids can be attached to the α-carboxyl group of D-glutamic acid in a manner similar to glycine in *M. luteus* (*lysodeikticus*). In the latter case glycine is added to the α-carboxyl group of D-glutamic acid in a reaction directly linked to adenosine triphosphate (ATP) hydrolysis (189).

(v) Interpeptide bridges containing a diamino acid reveal an interesting correlation between the mode of linkage and the configuration of these diamino acids. L-Diamino acids are always bound through their ω-amino group to

D-alanine and through their α-amino group to D-glutamic acid or glycine, whereas D-diamino acids are linked the other way round.

(vi) It is possible that a particular peptidoglycan contains peptide units with different amino acid composition. It was found in several organisms that L-lysine and L-ornithine occur in the same peptidoglycan at position 3 of the peptide subunits. The molar ratio of Lys/Orn varies among different, strains but the addition of lysine or ornithine to the growth medium did not significantly influence the ratio. In the interpeptide bridges sometimes L-alanine, L-serine, and glycine can partially replace each other.

PROPOSAL FOR A CONCISE SYSTEM FOR CHARACTERIZATION AND REPRESENTATION OF PEPTIDOGLYCAN TYPES

For the purpose of a simplified description of the peptidoglycan types, a concise abbreviation consisting of a characteristic fragment of the primary structure of the peptidoglycan will be used to represent the diverse peptidoglycans.

Peptidoglycan Group A

The abbreviations consist of the diamino acid occurring in position 3 of the peptide subunit and the interpeptide bridge, starting with the amino acid attached to the diamino acid (the C-terminus of the interpeptide bridge) as written in the figures. In so far as the interpeptide bridge is bound through its C-terminus to the ω-amino group of the diamino acid, this representation of the amino acid sequence is the reverse of the accepted convention in which peptides are written beginning with the N-terminus. If several identical amino acid residues occur in series in the interpeptide bridge, they are not written in full but are abbreviated by subscripts. If there is no interpeptide bridge, the diamino acid is given and the word "direct" is added. Some examples should illustrate this system.

Subgroup A1. The directly cross-linked, m-Dpm-containing peptidoglycan type (A1γ) is written: m-Dpm-direct. The directly cross-linked, L-Lys (A1α) and L-Orn (A1β) peptidoglycan types are abbreviated: L-Lys-direct and L-Orn-direct.

Subgroup A2. This subgroup contains no variations and hitherto only one peptidoglycan type: L-Lys-peptide subunit.

Subgroup A3. A few examples are chosen for illustration. (i) L-Lys occurs in position 3 of the peptide subunit, and the interpeptide bridge consists of three to four L-Ala residues (variation A3α, Fig. 15a): L-Lys-L-Ala$_{3-4}$. (ii) L-Orn occurs in position 3 of the peptide subunit, and the interpeptide bridge shows the sequence (starting from the N-terminus!) L-Ala-L-Thr-L-Ala-L-Ser (variation A3β, Fig. 24b): L-Orn-L-Ser-L-Ala-L-Thr-L-Ala. (iii) L-Lys occurs in position 3 of the peptide subunit, and the interpeptide bridge has the sequence L-Ser-L-Ala, but a minor part of L-Ala can be replaced by L-Ser (variation A3α, Fig. 23a): L-Lys-L-Ala(L-Ser)-L-Ser. In such cases always the amino acid occurring in smaller amounts is put in parentheses.

Subgroup A4. The dicarboxylic amino acids are always bound through their distal carboxyl groups (β or γ) in the interpeptide bridges, but for the purpose of simplification this is not specified. The amidation of the α-carboxyl groups is also not taken into consideration. If it is important for the taxonomic relevance of such a peptidoglycan type, it must be explicitly specified. Again a few examples should make this plain. (iv) L-Lys occurs in position 3 of the peptide subunit, and a D-β-Asp residue (or D-isoasparagine residue) connects two peptide subunits (variation A4α, Fig. 9): L-Lys-D-Asp. (v) L-Orn occurs in position 3 of the peptide subunit, and the interpeptide bridge consists of D-β-Asp-D-Ser (variation A4β, Fig. 24a): L-Orn-D-Ser-D-Asp. (vi) m-Dpm occurs in position 3 of the peptide subunit, and the interpeptide bridge consists of γ-D-Glu-D-Glu (variation A4γ, Fig. 17b): m-Dpm-D-Glu$_2$.

Peptidoglycan Group B

To describe peptidoglycan types of group B, the following concise system is used. In the first place the amino acid occurring in position 3 of the peptide subunit is written in brackets, followed by the amino acid occurring in position 2 (D-Glu) and the amino acid of the interpeptide bridge linked to the α-carboxyl group of D-Glu. The amino group of the diamino acid bound to the α-carboxyl group of D-Glu or Gly is not explicitly mentioned since the L-diamino acids are always linked through their α-amino groups, whereas the D-diamino acids are linked through their ω-amino groups.

The following examples should illustrate the abbreviation system of group B peptidoglycan types. (vii) L-Hsr occurs at position 3 and D-Glu in position 2 of the peptide subunit, and the peptide subunits are connected by D-Orn residues (variation B2β, Fig. 10b):

[L-Hsr]D-Glu-D-Orn. (viii) L-Glu occurs in position 3 and D-Glu in position 2 of the peptide subunit, and the interpeptide bridge consists of N^2-Gly-Gly-L-Lys (variation B1γ, Fig. 10b): [L-Glu]D-Glu-Gly_2-L-Lys.

In some cases, however, the concise system is not sufficient to include all the special properties of a peptidoglycan type. We have already mentioned the amidation of the dicarboxylic amino acids of the interpeptide bridge. Other deviations not included in the proposed system are the substitution of the α-carboxyl group of D-Glu in position 2 of the peptide subunit by an amino acid or the replacement of the common amino acid in position 1 of the peptide subunit (L-Ala in group A and Gly in group B) by another amino acid (e.g., L-Ser in group B). If the inclusion of these deviations is necessary for the distinction of peptidoglycan types, a short appendix should be given.

CORRELATION BETWEEN PEPTIDOGLYCAN TYPES AND TAXONOMIC GROUPING OF BACTERIA

Already in the early days of cell wall research, the amino acid composition of the peptidoglycan was considered to be useful as a taxonomic criterion at least within certain groups of bacteria (75, 173, 358, 428). During the past five years many more peptidoglycan types have been elucidated in detailed studies (109, 279, 333). Today the determination of the qualitative, or even the quantitative, amino acid composition is no longer sufficient to characterize the type of peptidoglycan unequivocally. As pointed out earlier (167) the amino acid composition of two strains may be identical, but the amino acid sequence or even the mode of cross-linkage may be quite different. Therefore, it is often necessary to establish the primary structure of the peptidoglycan. For the purpose of classification, however, the quantitative determination of the amino acid composition of the peptidoglycan is in many cases sufficient after the primary structure of the peptidoglycan has once been established. This kind of study is comparable to the studies on the amino acid sequences of homologous proteins. But there exists a basic difference between the genetic fixation of a protein structure and that of a peptidoglycan. The amino acid sequence of polypeptide chains of proteins is determined by the linear order of the nucleotide sequence in the corresponding gene. There is, therefore, a colinearity between these two sequences, and the former is the phenotypic expression of the latter. By comparing homologous polypeptide chains derived by mutations at one or more amino acid loci, it is possible to deduce the temporal sequence of base changes. This kind of deduction is impossible by comparing the different primary structures of peptidoglycans, since the mode of biosynthesis of this polymer is quite different from that of polypeptide chains and there is no colinearity between the nucleotide sequence and the amino acid sequence of the peptidoglycan. The latter is of course not the product of a single gene but the result of multiple gene actions. This may also be one of the reasons why the primary structure of the peptidoglycans is quite conservative and mutational changes are rare.

In the following section the distribution of the different peptidoglycan types among the various groups of bacteria will be discussed. For the classification of bacteria, the system used in *Bergey's Manual* (49) was generally chosen. For each genus or group of genera, a table will be presented which contains the various types of peptidoglycans occurring in this genus together with the names and numbers of strains studied so far. For simplification a concise system will be used for the representation of the diverse peptidoglycan types. This system has been discussed earlier in this article (*see above*): "Proposal for a concise system for characterization and representation of peptidoglycan types". In addition the variation indexes introduced in the preceding chapter will be also given.

Gram-Negative Bacteria

Studies on the amino acid composition and sequence of the peptidoglycans of different gram-negative bacteria have shown that there is no great variation within this group. A list of the species studied so far is given in Table 13. The peptidoglycans of these strains contain only the amino acids D-Glu, m-Dpm, and D,L-Ala in a molar ratio of about 1:1:1.5 to 2.0. Therefore, it is believed that all gram-negative bacteria belong to the same peptidoglycan type, viz., the directly cross-linked variation A1γ as shown in Fig. 6. In most cases only the amino acid composition of the peptidoglycan has been studied, whereas the primary structure has been established for only a few species such as *Escherichia coli* (394, 403, 405), *Brucella abortus* (234), *Aerobacter cloacae* (166a, 349), *Spirillum serpens* (204), *Proteus mirabilis* (190, 236, 238), *Vibrio fetus* (423a), and *Salmonella typhi* (377). Since the primary structure is so uniform, no further subdivision of the gram-

TABLE 13. *Amino acid composition of the peptidoglycans of various gram-negative bacteria*

Species	Molar ratio of amino acids			Reference
	Ala	Glu	m-Dpm	
Acetobacter xylinum	+ + +	+ +	- +	171
Aerobacter aerogenes	+ +	+ +	+ +	171
A. cloacae	1.5	1.0	1.0	349
Agrobacterium tumefaciens	+ +	+ +	+ +	171, 230
Azotobacter chroococcum	+ +	+ +	+ +	171
Bacterionema matruchotii	+ +	+ +	+ +	26
Bacteriodes convexus	2.17	1.0	0.97	393
Bdellovibrio bacteriovorus	2.15	1.0	1.1	383
Brucella abortus	+ -	+ +	- +	234
Caulobacter vibrioides	+ +	+ +	+ +	171
Escherichia coli	+ +	+ -	+ +	231
	1.5	1.0	0.7	404
Ferrobacillus ferrooxidans	2.33	1.0	1.0	400
Flavobacterium breve	+ -	+ +	+ +	171
Hydrogenomonas sp.	- +	+ -	+ +	171
Hyphomicrobium neptunium	1.84	1.0	1.04	164
Neisseria sp.	+ +	+ +	- +	125
Proteus mirabilis	1.78	1.0	0.93	190
Pseudomonas aeruginosa	+ +	+ +	+ +	63
P. fluorescens	- +	+ +	+ +	171
Salmonella gallinarium	- +	+ +	+ +	405
S. typhi	- +	+ +	+ +	377
Serratia marcescens	+ +	+ +	- +	171
Spirillum serpens	1.9	1.0	1.0	204
Thiobacillus thiooxidans	- +	+ -	+ +	66
Veillonella parvula	+ +	+ +	+ +	125
Vibrio fetus	1.62	1.0	0.91	423a

negative bacteria based on the types of peptidoglycan can be made. There may be variations in the extent of cross-linkage and in the degree of amidation of the non-peptide-linked carboxyl groups of the peptide subunit. Their taxonomic relevance, however, remains to be shown.

Gram-Positive Bacteria

The gram-positive bacteria reveal, contrary to the gram-negative organisms, a great variation in the composition and structural arrangement of their peptidoglycans.

Family Micrococcaceae. The classification of the gram-positive cocci was a point of controversy for a long time. It was believed that the staphylococci and the micrococci are rather closely related and they were even placed in one genus. However, the determination of the guanosine plus cytosine (GC) ratio of these cocci revealed that the micrococci and the staphylococci must be separated into strictly different genera, the genus *Staphylococcus* with a low GC ratio of 30 to 35% and the genus *Micrococcus* with a rather high GC content of about 70% (24, 40, 41, 203, 324). The determination of the cell wall composition of these organisms provided an additional indication for the separation of these two genera (177, 340). In addition, several other strains of "micrococci" were shown to be different in their GC content, in several physiological properties, and in cell wall composition and therefore have also to be considered as separate genera.

Genus Staphylococcus. Peptidoglycans with a high content of glycine are typical for staphylococci (18, 133, 205, 321, 327, 340, 368, 387, 390, 433, 434). Usually between 3 and 6 moles of glycine residues are found per mole of peptide subunit. Up to now three different peptidoglycan types (Fig. 12) have been detected in the genus *Staphylococcus* (340). Most of the strains called *S. aureus* contain a peptidoglycan cross-linked by penta- or hexaglycine bridges (Fig. 12a; 110, 113, 340, 385; Schleifer, *unpublished data*). Sometimes a small amount of glycine (0.1 to 0.25 mole/mole of glutamic acid) can be replaced by L-serine (340, 387). Only when the medium contains an unusually high level of L-Ser are Ser residues incorporated into the interpeptide bridge in significant amounts (334). Some strains of coagulase-positive staphylococci produce enterotoxin. To see if

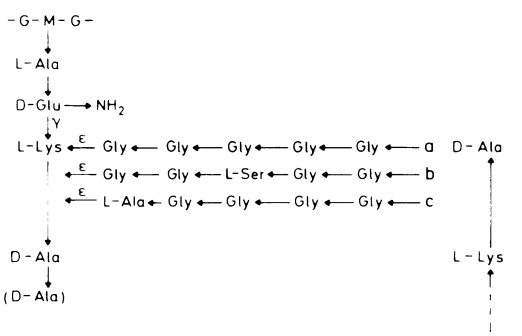

FIG. 12. *Fragments of the primary structures of peptidoglycans found in staphylococci (A3α). (a) S. aureus, L-Lys-Gly$_5$; (b) S. epidermidis, L-Lys-Gly$_4$, L-Ser; (c) S. epidermidis L-Lys-L-Ala-Gly$_4$.*

these strains constitute a separate group, we have studied the cell wall composition of 28 enterotoxin-producing strains (kindly supplied by H. J. Sinell, Berlin) with diverse pigmentation (white, yellow, orange). All of these strains showed the typical cell wall composition of *S. aureus* (Table 14). The peptidoglycans of most strains of *S. epidermidis* contain also pentaglycine or hexaglycine interpeptide bridges, but a significantly higher amount of Gly (0.5 to 1.7 moles/mole of Glu) is replaced by L-Ser (Fig. 12b; 346, 387). A few other coagulase-negative strains reveal a peptidoglycan type in which the interpeptide bridge consists of a tetraglycyl-L-alanine peptide (Fig. 12c; 344).

Besides peptidoglycan, protein and teichoic acid are the other main cell wall polymers of staphylococci (335a). The distribution of the different types of teichoic acid among staphylococci is another chemotaxonomic criterion (85, 86) which is also important for the different serological behavior of staphylococci (275) and the adsorption of phages (62).

Table 14 compiles our studies on the distribution of peptidoglycan types and teichoic acid types within the genus *Staphylococcus*. The term "unusual teichoic acids" includes all polymers similar to teichoic acid which do not contain a typical alditol phosphate backbone but a glycosyl-phosphate-alditol-phosphate backbone (15) or a glycosyl phosphate backbone (13, 281). The biosynthesis of these "unusual teichoic acids" (51, 94) is probably different from that of usual teichoic acids.

From the different chemical structures of the two main cell wall polymers of staphylococci, one can distinguish at least five different groups (Table 14). The two main groups are the typical *S. aureus* strains containing a ribitol teichoic acid and a peptidoglycan with little or no serine and the typical *S. epidermidis* strains with glycerol teichoic acid in their walls and a peptidoglycan with a relatively high serine content. The other three groups are represented only by a few strains, which are also called *S. epidermidis*. This indicates heterogeneity within the "species" *S. epidermidis*.

Among the strains which were delineated as micrococci, some were found with a peptidoglycan of staphylococcal type (335a). These strains are listed in Table 15. All of these strains are able to produce acid from glucose under anaerobic conditions, although in some cases in only low amounts. On the basis of peptidoglycan types and physiological properties, all of these strains have to be considered as staphylococci. Only in the case of *M. caseolyticus* 116 is the GC content surprisingly high (418).

An intermediate group of gram-positive cocci was studied by Mortensen and Kocur (262).

TABLE 14. *Distribution of peptidoglycan and teichoic acid types in the genus Staphylococcus*[a]

Peptidoglycan type	Teichoic acid type	No. of strains	Culture collection strain no.
Lys-Gly_{5-6} (only traces of L-Ser)	Ribitol	46	*S. aureus* ATCC 12600, 14458, 15234 *S. aureus* CCM 2317 *S. aureus* H,[b] 3528, *S. aureus* Copenhagen[c] *S. aureus* Oxford 511 28 Entertoxin-producing strains,[d] 10 own isolates
	Glycerol	4	*S. epidermidis* CCM 901, 2274, 2340, 2433
Lys-Gly_4, L-$Ser_{0.5-1.5}$	Glycerol	26	*S. epidermidis* ATCC 155, 12228, 14990, 27992 *S. epidermidis* Korman 968A,[e] 2476,[e] 1449[e] *S. epidermidis* Texas 26,[f] 10 own isolates
	Unusual teichoic acid (see text)	3	*S. epidermidis* NCTC 2102[g] *S. epidermidis* CCM 2368, 2435
Lys-L-Ala-Gly_4	Unusuall teichoic acid	3	*S. epidermidis* I 3,[h] two own isolates

[a] All types belong to variation A3α.

[b-h] Cell wall composition and peptidoglycan type of all strains were determined in our laboratory (177, 340; unpublished results). A few strains were studied earlier in other laboratories: *b*, teichoic acid (29); *c*, peptidoglycan and teichoic acid (110, 111, 113); *d*, strains obtained by H. J. Sinell, Berlin; *e*, teichoic acid (424); *f*, peptidoglycan and teichoic acid (385, 387); *g*, teichoic acid (13); *h*, teichoic acid (15).

Staphylococci are usually separated from micrococci by their ability to produce acid from glucose under anaerobic conditions (371). These intermediate strains, however, produce only little, if any, acid from glucose under anaerobic conditions. Thus, the method proposed by the Subcommittee failed to classify this group of cocci. One possible method of grouping these strains was by the determination of their GC content (262). According to their low GC content, four of these strains are staphylococci, whereas the other three strains reveal a rather high GC ratio similar to micrococci. As shown in Table 16 there is an excellent agreement between the GC content and the peptidoglycan types. All strains with a low GC content, typical for staphylococci, also possess a typical staphylococcal peptidoglycan. On the other hand, the strains with a high GC ratio typical for micrococci contain a peptidoglycan with a tri-L-alanine interpeptide bridge as found among *M. roseus* and *M. lactis* (*see below*).

Genus Micrococcus. The micrococci are distinguished from staphylococci by their high GC values, namely 66 to 75%. With regard to their peptidoglycan structure, they can be divided in two main groups. Group I includes only the species *M. luteus* and some related organisms (Table 17), including most "aerobic *Sarcina*" strains. It contains an unique peptidoglycan type, namely subgroup A2 (Fig. 7), in which a polymerized peptide subunit is involved in the cross-linkage. Group II contains peptidoglycan types of variation A3α usually with a tri- or tetra-alanine peptide as interpeptide bridge (Fig. 13a). This type is widely distributed and has been also found among streptococci and corynebacteria. Within the micrococci this type of peptidoglycan is restricted to *M. roseus*, *M. varians*, and some related organisms with different names (Table 17). A similar separation of the genus *Micrococcus* into two groups can be obtained by genetic analysis (195). A collaborative study (K. H. Schleifer, W. E. Kloos, and A. Moore, Taxonomic status of *Micrococcus luteus* [Schroter] Cohn: correlation between peptidoglycan type and genetic compatibility, Int. J. System. Bacteriol. **22**:224–227 has shown that only the organisms possessing peptidoglycan types of subgroup A2 can participate in transformation of *M. luteus* (*lysodeikticus*) or *Sarcina lutea* ATCC 292. Organisms with other peptidoglycan types are unable to perform genetic exchange with these two strains. This is an indication that the separation of *M. luteus* from *M. roseus* and *M. varians* based on their different peptidoglycan types is also supported by their different genetic behavior. Moreover, these differences in the peptidoglycan structure of *M. luteus*, *M. roseus*, and other micrococci are correlated with different hydrocarbon distribution patterns (261, 392).

Two strains of *M. roseus* reveal a different peptidoglycan type. They contain instead of the

TABLE 15. *Strains called Micrococcus containing peptidoglycan types found in Staphylococcus*[a]

Name and strain no.	% GC	Peptidoglycan type
Micrococcus caseolyticus ATCC 13548	35	L-Lys-Gly$_{4-5}$, L-Ser
M. caseolyticus 116 (from M. R. J. Salton)	44.4	L-Lys-Gly$_{4-5}$, L-Ser
M. candicans ATCC 14852	30	L-Lys-Gly$_{4-5}$, L-Ser
M. naucinus ATCC 15935	30	L-Lys-Gly$_5$
M. vernae ATCC 10209	ND	L-Lys-Gly$_{4-5}$, L-Ser

[a] ND, Not determined. All strains produce acid from glucose under anaerobic conditions.

TABLE 16. *Correlation between type of peptidoglycan and GC ratio of gram-positive cocci of uncertain taxonomic position*

Name and strain no.	%GC	*p*H values of glucose medium after anaerobic incubation	Peptidoglycan type
Staphylococcus saprophyticus NCTC 7293	31.6	5.7	L-Lys-Gly$_{4-5}$, L-Ser
S. saprophyticus NCTC 7612	30.8	5.7	L-Lys-Gly$_{4-5}$, L-Ser
S. lactis CCM 1400	30.2	6.4	L-Lys-Gly$_5$
S. lactis NCTC 189	32.8	6.5	L-Lys-Gly$_5$
S. lactis NCTC 7564	69.0	5.3	L-Lys-Ala$_{3-4}$
Micrococcus sp. CCM 825	69.5[b]	6.8	L-Lys-Ala$_{3-4}$
Micrococcus sp. CCM 740	58.0[b]	6.6	L-Lys-Ala$_{3-4}$

[a] The *p*H values were determined by Mortensen and Kocur (262); the peptidoglycan types were determined by Schleifer (335a).

[b] Data by Bohacek et al. (47), remaining data on GC are by Silvestri and Hill (357).

TABLE 17. *Peptidoglycan types of various micrococci*

Variation	Type	Species	Strain
A2	L-Lys-peptide subunit	*Micrococcus luteus*	CCM 149, 169, 247, 248, 265, 266, 309, 355, 370, 409, 622, 840, 851, 852, 853, 1335, 1569, 1674, 2266.
		M. citreus	R 266 (Pasteur Institute, Paris)[a]
		M. flavus	ATCC 400, 10240; strain 53160 (Pasteur Institute, Paris)[a]
		M. lysodeikticus	ATCC 12698, NCTC 2655[a]
		M. sodonensis	ATCC 11880[b]
		Sarcina flava	ATCC 147, 540
		S. lutea	ATCC 272, 381, 382, 9622, 10054, 10773, 15220, R 262 (Pasteur Institute, Paris)[a]
		S. subflava	ATCC 7468
A3α	L-Lys-L-Ala_{3-4}	*M. roseus*	ATCC 144, 177, 178, 179, 185, 412, 416, 418, 516, 534, 9815; CCM 189, 347, 570, 679, 908, 1145, 1679, 2179, 2180, strain $Thr^{(-)c}$
		M. flavoroseus	ATCC 397[d]
		M. lactis	CCM 268, 418, 1395, 1411, 1414, 2139, 2141, 2189, 2431
		M. luteus	CCM 310
		M. varians	ATCC 399, 19099, 19100; CCM 2253
		M. conglomeratus	ATCC 401, CCM 825, 884
		M. salivarius	ATCC 14344
		M. pulcher	ATCC15936
		S. lutea	ATCC 383, 533, 9341
		S. aurantiacus	ATCC 146
		S. erythromyxa	ATCC 187
	L-Lys-Thr-Ala_3	*M. roseus*	CCM 2131, 2390; R 27 (Pasteur Institute, Paris)[c]

[a-d] Peptidoglycan types were determined in our laboratory (177, 340; *unpublished studies*) with the exception of: *a*, determined by Campbell et al. (59); *b*, also determined by Johnson and Campbell (163); *c*, determined by Petit et al. (293); *d*, inadvertently presented as "L-Lys-peptide subunit" type (340).

normal tri- or tetra-alanine interpeptide bridges an interpeptide bridge consisting of a tri-L-alanyl-L-threonine, peptide (Fig. 13b). These strains are also physiologically distinct from normal *M. roseus* strains (201) and may represent a new species. The same peptidoglycan was described by Petit et al. (293) in a strain of *M. roseus*. They thought, however, that the L-Thr-containing strain is a typical *M. roseus* and the other strain lacking L-Thr and containing only L-Ala residues in the interpeptide bridge is a mutant. But our studies have clearly demonstrated that the strains containing only L-Ala residues in the interpeptide bridges are the genuine *M. roseus*.

Some unusual peptidoglycan types have been elucidated in micrococci of doubtful taxonomic position (Table 18). In *M. luteus* ATCC 398 and *M. freudenreichii* ATCC 407, the peptides γ-L-Glu-Gly and γ-L-Glu-L-Ala cross-link the peptide subunits (Fig. 14a and b). Both strains have a distinctly lower GC content than the usual micrococci. The GC content of *M. luteus* ATCC 398 has been determined to be 66% (324) and that of *M. freudenreichii* to be 58 to 59% (37). *M. luteus* ATCC 398 also has a different menaquinone pattern (162) and shows no genetic exchange with a typical *M. luteus* (195).

The peptidoglycan of *M. radiodurans* contains L-Orn instead of L-Lys (427), and the interpeptide bridge consists of di- or triglycine peptide (Fig. 14f; 109, 340). *M. radiodurans* also differs from typical micrococci by the occur-

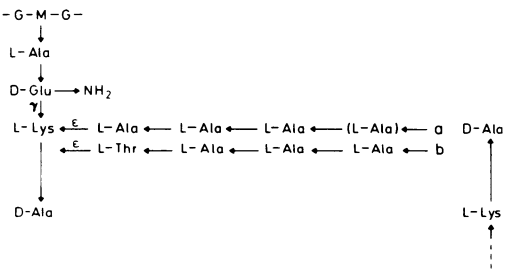

FIG. 13. *Fragments of the primary structures of peptidoglycans (A3α) found in various micrococci listed in Table 17. (a) L-Lys-L-*Ala_{3-4}*; (b) L-Lys-L-Thr-L-*Ala_3.

rence of significant amounts of a lipoprotein-polysaccharide complex as is typical for gram-negative bacteria. This latter character is the reason why this organism is excluded from the genus *Micrococcus* (31, 32). The unique peptidoglycan type supports this exclusion.

A few gram-positive cocci contain Ser in the interpeptide bridge of the peptidoglycan (Fig. 14c and d), either combined with Ala or with D-Glu. These organisms differ from typical micrococci by their significant lower GC ratio (Table 18). They may be excluded from the genus *Micrococcus*.

The peptidoglycan of two other gram-positive cocci is similar to that of staphylococci. The interpeptide bridge contains six Gly and one L-Ala residues. But in contrast to the peptidoglycan of some staphylococci in which L-Ala occurs at the C-terminus of the interpeptide bridge (Fig. 12c), L-Ala is the N-terminus of the interpeptide bridge in these two organisms (Fig. 14e). As in staphylococci the cell walls of both strains show a rather high phosphate content, but the organisms do not produce acid from glucose under anaerobic conditions. The GC content of 49% (203) indicates a separate position (distinct from either *Micrococcus* or *Staphylococcus*).

M. rhenanus var. *miyamizu* CCM 2142 contains L,L-Dpm instead of L-Lys in its peptide subunit (Fig. 15a). This type of peptidoglycan and the high GC content indicate that the organism may belong to the genus *Arthrobacter* (*see below*).

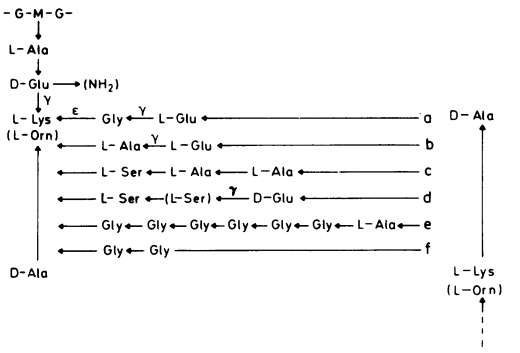

FIG. 14. *Fragments of the primary structures of peptidoglycans found in micrococci of doubtful taxonomic position (A3α, A3β, A4α) listed in Table 18. (a) L-Lys-Gly-L-Glu; (b) L-Lys-L-Ala-L-Glu; (c) L-Lys-L-Ser-L-Ala$_2$; (d) L-Lys-L-Ser$_{(2)}$-D-Glu; (e) L-Lys-Gly$_6$-L-Ala; (f) L-Orn-Gly$_2$.*

A rather unique peptidoglycan type was found among a small group of gram-positive cocci (Table 18). This peptidoglycan contains m-Dpm, and the interpeptide bridge is made up of two D-Glu residues (Fig. 15b). With the exception of the peptidoglycan of *S. lactis* CCM 2432, the α-carboxyl group of D-Glu of the peptide subunit is substituted by glycine amide. Almost the same type of peptidoglycan was found among some *Arthrobacter* sp. (*see below*). The only difference is that the peptidoglycan of these arthrobacters contains an additional amide group. The carboxyl group of m-Dpm not involved in the peptide linkage is

TABLE 18. *Peptidoglycan types of micrococci of doubtful taxonomic position*

Variation	Type	Fig.	Strain	Reference	%GC	Reference
A3α	L-Lys-L-Ser-L-Ala$_2$	14c	*Sarcina oliva* CCM 250	Schleifer et al., *manuscript in preparation*	54.2	203
A3α	L-Lys-Gly$_6$-L-Ala	14e	*Micrococcus* CCM 168		49.3	203
		14e	*Micrococcus* CCM 1405		49.3	203
A3β	L-Orn-Gly$_2$	14f	*M. radiodurans* ATCC 13939	109, 340	66	203
A3γ	L,L-Dpm-Gly	15a	*M. rhenanus* var. *miyamizu* CCM 2142	Schleifer et al., *manuscript in preparation*	70.5	203
A4α	L-Lys-Gly-L-Glu	14a	*M. luteus* ATCC 398	271	66	324
A4α	L-Lys-L-Ala-L-Glu	14b	*M. freudenreichii* ATCC 407	271	59	24
A4α	L-Lys-L-Ser$_2$-D-Glu	14d	*M. nishinomiyaensis* CCM 2140	Schleifer et al., *manuscript in preparation*	67.8	203
A4α	L-Lys-L-Ser-D-Glu	14d	*M. cyaneus* CCM 856		63.0	203
A4γ	m-Dpm-D-Glu$_2$	15b	*M. varians* NCTC 7281	39	72.4	418
			M. luteus CCM 2136, 2137	39	70.6 71.2	203
		15b	*M. conglomeratus* CCM 2134	39	71.4	203
		15b	2135 (ATCC 19101, 19102)	39	72.0	203
		15b	*S. lactis* CCM 2432 (NCTC 7567)	39	71.2	203

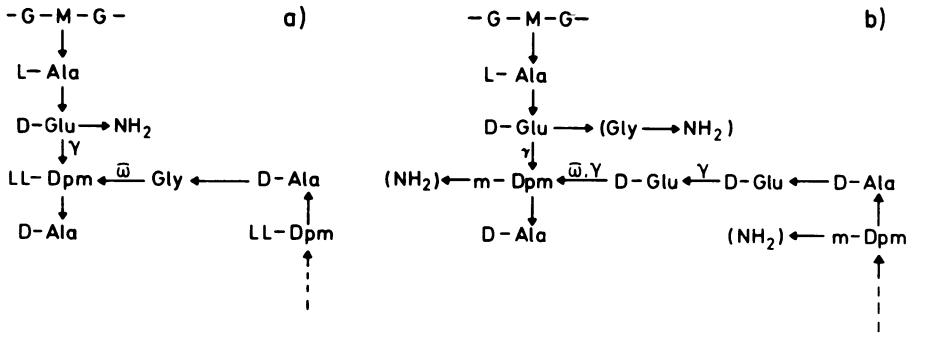

FIG. 15. *Fragments of the primary structures of Dpm-containing peptidoglycans of micrococci of doubtful taxonomic position (A3γ and A4γ) listed in Table 18. (a) L, L-Dpm-Gly; (b) m-Dpm-D-Glu₂.*

probably amidated there. The occurrence of this very rare type of peptidoglycan among a small group of bacteria may indicate a connection between this group of micrococci and certain arthrobacters. Although the GC ratio of 70 to 72% (203) is in the same range as that of the "true" micrococci, the strains are also different from the genus *Micrococcus* by physiological criteria (Kocur, *personal communication*). In our opinion these strains form a distinct taxon.

Micrococcus mucilaginosus. In 1967 Gordon reisolated three strains of *Staphylococcus salivarius* and determined their GC ratio to be 56 to 60% (123). Bergan et al. (36) isolated identical strains, transferred them to the genus *Micrococcus*, and employed the epithet *mucilaginosus*. *M. mucilaginosus* strains form a rather homogeneous group. The peptidoglycan type of seven strains has been studied in our laboratory. In all cases the cross-linkage is mediated by only one amino acid residue (Fig. 16), namely L-Ala, L-Ser, or Gly, whereby one amino acid can be replaced in part or completely by another one. The peptidoglycan type of *M. mucilaginosus* is quite distinct from that of staphylococci and typical micrococci (*M. luteus*, *M. roseus*, *M. varians*).

Genus Planococcus. A group of marine motile cocci has been recently characterized by a GC content of 40 to 51% (42, 203) and transferred to the genus *Planococcus* Migula (202). All of the strains belong to the same peptidoglycan type (340; Fig. 17a) containing one D-Glu residue as interpeptide bridge. This peptidoglycan type not found in other gram-positive, catalase-positive cocci supports the separation of the planococci from all the other "micrococci."

Sporosarcina ureae. *Sporosarcina ureae* is a unique organism among the gram-positi

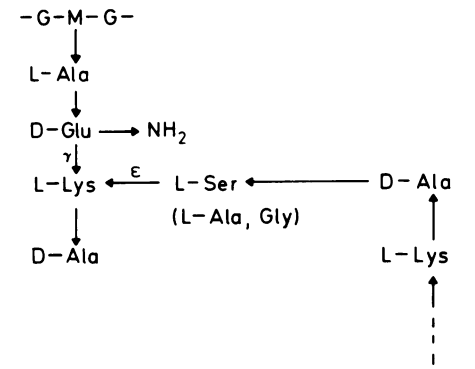

FIG. 16. *Fragments of the primary structure of the peptidoglycan of M. mucilaginosus (A3α). Amino acids in parentheses may replace L-Ser partially or completely.*

cocci. It is, on the one hand, similar to the genus *Planococcus* in its shape, motility, and GC content (43) and, on the other hand, to *Bacillus pasteurii* in its ability to sporulate, physiological properties, and GC content (43). The peptidoglycans of seven strains of *S. ureae* have been studied and all strains revealed the same peptidoglycan type (Ranftl, Ph.D. thesis, Technical University, Munich, 1972). The interpeptide bridge contains glycine and, like the peptidoglycan of the planococci, a γ-linked D-Glu (Fig. 17b). Together with the other criteria mentioned above the unique peptidoglycan of *S. ureae* justifies the transfer of this species to the separate genus *Sporosarcina*. This confirms also the studies of Herdon and Bott (139) which showed that the deoxyribonucleic acid (DNA)-RNA homology between

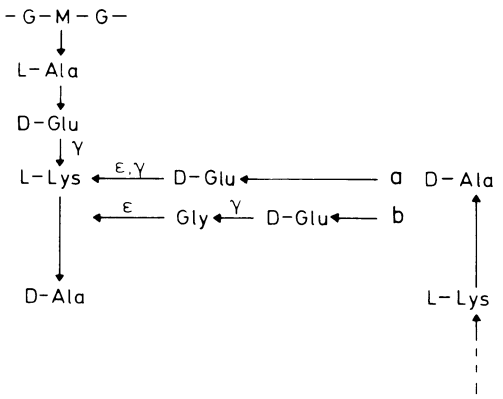

FIG. 17. *Fragments of the primary structures of peptidoglycans of Planococcus and Sporosarcina (A4α). (a) Planococcus sp.: CCM 316, 1849, 2069, 2104, 2387, 2388, 2389, 2414, 2415, 2416;* L-*Lys-*D-*Glu. (b) S. ureae: ATCC 6473, CCM 380, 752, 860, 981, 1732, 1743;* L-*Lys-Gly-*D-*Glu.*

strains of *Sporosarcina* and certain bacilli, especially *B. pasteurii*, is better than between *Sporosarcina* and micrococci.

Genus Sarcina. The low GC content (28–30%) of the anaerobic organisms *S. maxima* and *S. ventriculi* indicates that these organisms are phylogenetically remote from aerobic strains called *Sarcina* with their high GC content of about 70%. On the basis of these findings Canale-Parola (60) concluded that only the anaerobic, sugar-fermenting species *S. ventriculi* and *S. maxima* should be considered as *Sarcina*. The peptidoglycans of two strains of *S. maxima* and two strains of *S. ventriculi* were studied by Kandler et al. (178), and it was found that they belonged to the same peptidoglycan type. All strains contained L, L-Dpm and were cross-linked by a glycine residue (Fig. 15a). This finding supports the separation of the anaerobic from the aerobic tetrad-forming organisms.

Genus Aerococcus. A group of mostly catalase-negative, gram-positive cocci with a low GC content of 35 to 43% was described by Bohacek et al. (44) and Evans and Schultes (100a). They are called *A. viridans* and *A. catalasicus*; the latter are catalase-positive. The amino acid sequence of the peptidoglycan of *A. viridans* strains Evans 207 and ATCC 11563 was studied in detail (268). In contrast to all the other peptidoglycan types found in micrococci, this type contains no interpeptide bridge. The carboxyl group of D-Ala is directly linked to the ε-amino group of L-Lys of an adjacent peptide subunit (Fig. 18). Comparison of the peptide patterns of partial acid hydrolysates of cell walls of 33 other strains of aerococci (kindly supplied by Baird-Parker, Evans, and Kocur) and the determination of the molar ratios of the amino acids of these cell walls indicated that they contain the same directly cross-linked peptidoglycan variation A1α as strains Evans 207 and ATCC 11563. The degree of cross-linkage is very low, since 70% of the lysine residues reveal an unsubstituted ε-amino group. In addition most of D-Ala is missing. Therefore, the free peptide subunits probably consist of tripeptides with a C-terminal L-Lys. The α-carboxyl group of D-Glu is amidated in a part of the peptide subunits. The uniformity of the peptidoglycan type together with the low GC content of all strains supports their unification within a separate genus.

Genus Gaffkya. The genus *Gaffkya* is a rather heterogeneous group. The strains of *Gaffkya homari* show a GC content similar to that of the aerococci, and DNA-hybridization studies also confirmed the relationship between *Gaffkya homari* and aerococci (350, 351). The same is true for the peptidoglycan type. *Gaffkya homari* only differs from the aerococci by a relative high alanine content in its walls (2 moles per mole of glutamic acid), but the cross-linkage is the same as in aerococci (268). The identical peptidoglycan type supports, together with the other criteria mentioned above, the transfer of *Gaffkya homari* to the genus *Aerococcus* as suggested earlier (88, 351).

A diverse array of bacteria have been labeled

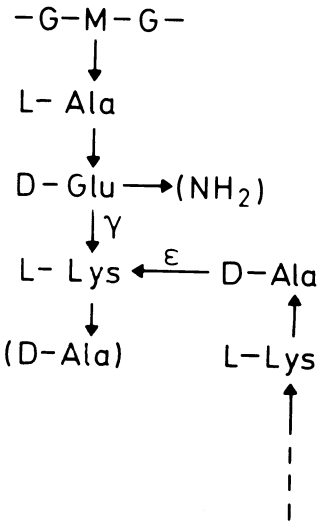

FIG. 18. *Fragment of the primary structure of the directly cross-linked peptidoglycan (A1β) of aerococci and streptococci (listed in Tables 20 and 22).* L-*Lys-direct. A. viridans ATCC 11563; strains from J. Evans (Raleigh, N.C.): 3, 53, 95, 132, 146, 165, and 201; A. catalasicus: CCM 2437, 2451, 2452; Gaffkya homari: ATCC 10400.*

Gaffkya tetragena. The peptidoglycan of *Gaffkya tetragena* ATCC 10875 was studied in our laboratory. The peptidoglycan type is like that of *S. epidermidis* (Fig. 14b), and the GC content is also typical for a *Staphylococcus* (350).

Micrococcus cryophilus. Cell walls were prepared from freeze-dried cells of *M. cryophilus* CCM 900 (ATCC 12226) kindly supplied by M. Kocur (Brno). Analysis of these cell walls revealed a directly cross-linked, m-Dpm-containing peptidoglycan (177). This peptidoglycan type is not found in other gram-positive, catalase-positive cocci. But *M. cryophilus* is known to be very unusual in many respects. The fine structure of the cell wall is intermediate between that of gram-negative and gram-positive bacteria (244), and the GC content is only 41% (41, 203). Thus, *M. cryophilus* is certainly different from typical micrococci and has to be excluded from the genus *Micrococcus*.

Micrococcus morrhuae. *M. morrhuae* is an obligate halophilic organism with a GC content varying in different strains from 57.1 to 61.4% (42). The taxonomic position of this species is uncertain. Venkataraman and Sreenivasen (396) recommended that it should be placed in a separate genus named *Halococcus*. Studies in our laboratory have shown that these organisms contain no peptidoglycan (177), despite the very thick cell wall. According to Larsen (215) these cell walls consist of polysaccharide. The absence of a peptidoglycan supports the exclusion of this species from the genus *Micrococcus*. The grouping of the family *Micrococcaceae* according to GC content and peptidoglycan type is given in Table 19.

Family Lactobacillaceae

The family *Lactobacillaceae* comprises microaerophilic or anaerobic cocci and rods which produce lactic acid from carbohydrates by homo- or heterofermentation. They can be subdivided into seven genera: *Diplococcus*, *Streptococcus*, *Peptostreptococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Bifidobacterium*.

Genus Diplococcus. The chemical composition of the cell walls of *Diplococcus pneumoniae* has been studied by several groups. In particular the composition and structure of the antigenic C-polysaccharide were determined in detail (53, 54, 124, 223, 263). The C-polysaccharide is a choline-containing teichoic acid and represents a major structural component of the cell wall. Little, however, is known about the structure of the peptidoglycan. A tentative model was proposed by Mosser and Tomasz (263). They assumed that the peptide subunits are cross-linked by polymerized peptide subunits (subgroup A2) as in the peptidoglycan of *M. luteus* but there is yet no proof.

Genus Streptococcus. Physiological and immunological tests have usually been applied for the classification of streptococci at the subgroup and species level. The so-called group (Lancefield groups A–S) and type antigens can be detected by agglutination tests with whole cells or precipitin reactions with extracted

TABLE 19. *Grouping of the family Micrococcaceae according to the GC ratios and peptidoglycan types*

Genus	Typical species or strain	%GC content[a]	Peptidoglycan type
Micrococcus	*M. roseus*, *M. varians*	69–73	L-Lys-L-Ala$_{3-4}$
Micrococcus	*M. luteus*	70–75	L-Lys-peptide subunit
Not defined	*M. conglomeratus*	70–72	m-Dpm-D-Glu$_2$
Not defined	*M. nishinomiyaensis* CCM 2140 *M. cyaneus* CCM 856	63–67	L-Lys-L-Ser$_{1-2}$-D-Glu
Not defined	*M. luteus* ATCC 398	66	L-Lys-Gly-L-Glu
Not defined	*M. freudenreichii* ATCC 407	59	L-Lys-L-Ala-L-Glu
Halococcus	*M. morrhuae*	57–61	No peptidoglycan
Not defined	*M. mucilaginosus*	56–60	L-Lys-L-Ala(Gly or L-Ser)
Not defined	*Sarcina oliva* CCM 250	54.2	L-Lys-L-Ser-L-Ala$_2$
Not defined	*Micrococcus* spp. CCM 168 and 1405	49.3	L-Lys-Gly$_6$-L-Ala
Planococcus	*Planococcus* sp.	40–50	L-Lys-D-Glu
Sporosarcina	*Sporosarcina ureae*	45	L-Lys-Gly-D-Glu
Not defined	*M. cryophilus* CCM 900	41	m-Dpm-direct
Aerococcus	*A. viridans*	35–43	L-Lys-direct
Staphylococcus	*S. aureus*	30–35	L-Lys-Gly$_{5-6}$
	S. epidermidis		L-Lys-L-Ala-Gly$_4$, L-Lys-Gly$_4$, L-Ser$_{0.5-1.5}$
Sarcina	*S. ventriculi*	28–30	L, L-Dpm-Gly

[a] According to Kocur et al. (203).

antigens. These immunological tests are based on the presence of specific chemical structures on or in the outermost surface layers of the cell (207). Thus, the traditional classification of the streptococci employs indirectly the differences in the chemical structure of the cell wall as a distinguishing feature. Studies on the amino acid composition and amino acid sequence of streptococcal peptidoglycans have revealed 12 different types. The distribution of these peptidoglycan types in the genus *Streptococcus* is compiled in Table 20. Most of the peptidoglycans found in streptococci belong to variation A3α with L-Lys in position 3 of the peptide subunit and interpeptide bridges consisting of monocarboxylic L-amino acids or glycine, or both. A few of the streptococci contain a peptidoglycan cross-linked by a dicarboxylic amino acid (variation A4α) and that of others is directly cross-linked (variation A1α).

A large number of different streptococci contain a peptidoglycan cross-linked by interpeptide bridges consisting of L-alanyl oligopeptides. The length of these chains varies from one to four alanine residues. These types of peptidoglycan have been found in streptococci belonging to groups A, A-variant, C, C-variant, D (*S. faecalis*), E, F, G, H, K, L, M, P, U, and *S. thermophilus*.

In a few other species one of the Ala residues of the interpeptide bridge is replaced by L-Ser or Gly. The peptidoglycans of *S. agalacticae* (group B), of one strain of group K, and of one strain of group C contain in part L-Ser residues instead of L-Ala residues in the interpeptide bridge. In *Streptococcus* sp. of group L and *S. viridans* IV, one L-Ala residue is replaced by a Gly residue. Several types of peptidoglycans occurring among streptococci reveal as an additional amino acid L-Thr which is bound to the ϵ-amino group of L-Lys. The complete interpeptide bridges consist of dipeptides which contain, besides L-Thr, the amino acids Gly, L-Ala, or L-Ala/L-Ser. The strains of *S. mutans* and some strains of *S. equinus* show the peptidoglycan type Lys-L-Thr-L-Ala (Fig. 19a). This type was first described by Schleifer and Kandler (336) for a *Streptococcus* which was most probably wrongly delineated as *S. cremoris* (Hladny, Ph.D. thesis, Technical University, Munich, 1971). Almost the same type was found in *S. bovis* (179), in some strains of *S. equinus*, and in a strain of *S. milleri* (14a); the only difference is that some of the Ala residues in the interpeptide bridges are replaced by Ser. In one strain of *S. equinus*, in two strains of *S. milleri*, and in *S. salivarius* II (not reacting with antiserum against group K), the interpeptide bridges consist of Gly-L-Thr peptides (Fig. 19b; 141a).

The L-Lys-D-Asp type is found in *S. faecium*, *Streptococcus* sp. (group Q), *S. lactis*, and *S. cremoris* (group N).

The directly cross-linked peptidoglycan (A1α) occurs among strains of groups K, O, R, and *S. viridans* I, II, and IV.

From a comparison of the serological grouping and the occurrence of peptidoglycan types, it is evident that most of the serological groups belong to one type of peptidoglycan, the only exceptions being groups D and K. Group D is a very heterogeneous group and the analysis of the peptidoglycan confirmed the separation of this group, on the basis of physiological tests (87), into five different species. By determining the qualitative amino acid composition of the cell walls, it is easily possible to distinguish between *S. faecalis* and *S. faecium* (175) and also between these two species on the one hand and *S. bovis* and *S. equinus* on the other hand.

Strains of *S. salivarius* and other *Streptococcus* spp. belonging to group K can be distinguished not only by their peptidoglycan types but also by their ability to form levan as seen in Table 21. It is likely that group K will have to be subdivided into several species like group D, but many more strains need to be studied.

S. sanguis I and *S. sanguis* II can be separated according to their reactions with antiserum to group H and their physiological and different cell wall compositions. It was suggested that *S. sanguis* II should be separated from *S. sanguis* I and reclassified as *S. pseudosanguis* (Hladny, Ph.D. thesis, Technical University, Munich, 1971). Most of the strains of *S. viridans* reveal the directly cross-linked, L-Lys-containing peptidoglycan type. Only the strain of *S. viridans* IV is different. This suggests that it may be necessary to study more strains of *S. viridans* to establish this distinction between *S. viridans* IV and the other streptococci of the viridans group.

The grouping of streptococci according to peptidoglycan types can shed some light on the relationships among various species or serological groups. A closely related cluster may be formed by *S. pyogenes* (group A), *S. equisimilis* (group C), and *Streptococcus* sp. (group G). They behave almost identically in their physiological properties and in their human pathogenicity, and they also contain quite similar peptidoglycan types (Table 22). A similar relationship can be observed between *S. uberis* (group E), *Streptococcus* sp. (group P), and

TABLE 20. *Distribution of peptidoglycan types (variations A3α and A4α) in the genus Streptococcus*

Species	Serological group	No. of strains	Culture collection[a] strain no.	Type of peptidoglycan	Fig.	Reference
S. pyogenes	A	43	Kiel 314, 4875, 25879	L-Lys-L-Ala$_{2-3}$	19a	75, 137, 140, 159, 207, 208, 246, 266, 358; Hladny and Kandler[b]
—	A variant	3		L-Lys-L-Ala$_{2-3}$	19a	38, 184, 207; Schleifer, *unpublished*
S. agalacticae	B	6	Kiel 13164, 25885, 25886	L-Lys-L-Ala$_2$ (L-Ser)	19b	75, 184, 358; Hladny and Kandler
S. zooepidemicum	C		Kiel 1300, 26176			75, 207, 209, 246; Hladny and Kandler
S. equi	C		Kiel 69, 21352			
S. dysgalactiae	C	14	Kiel 2200, 25922	L-Lys-L-Ala$_{2-3}$	19a	
S. equisimilis	C		Kiel 39, 25903, 26193			
...	C variant	1				184
S. faecalis	D	34	22 ATCC strains, 12 strains from Kiel	L-Lys-L-Ala$_{2-3}$	19a	75, 154, 155, 175, 326, 327; Hladny and Kandler[b]
S. faecium	D	22	6 ATCC strains, 16 strains from Kiel	L-Lys-D-Asp	9	109, 153, 155, 175, 391
S. faecium var. *durans*	D	5	3 ATCC strains	L-Lys-D-Asp	9	175
S. bovis	D	5	Kiel 25793, 25794	L-Lys-L-Thr-L-Ala (L-Ser)	20a	179, 141a
S. equinus	D	10		L-Lys-L-Thr-L-Ala (L-Ser)	20a	141a
S. equinus	D	1		L-Lys-L-Thr-Gly	20b	141a
S. uberis	E	4	Kiel 13478, 21342	L-Lys-L-Ala$_{3-4}$	13a	75, 7358; Hladny and Kandler
Streptococcus sp.	F	5	Kiel 12678; NCTC 5389	L-Lys-L-Ala$_{1-2}$	19a	75, 184, 185; Hladny and Kandler
Streptococcus sp.	G	5	Kiel 20793	L-Lys-L-Ala$_{1-2}$	19a	75, 184, 207, 358, Hladny and Kandler
Streptococcus sp.	G	1	Kiel 25926	L-Lys-L-Ala$_3$ (L-Ser)	19c	Hladny and Kandler
S. sanguis	H	2	Kiel 21384, 25835	L-Lys-L-Ala$_{2-3}$	19a	Hladny and Kandler
S. sanguis II (*pseudosanguis*)		2	Kiel 25826, NCTC 7864	L-Lys-direct	18	Hladny and Kandler
Streptococcus sp.	K	2	Kiel 226, 13272	L-Lys-direct	18	Hladny and Kandler
Streptococcus sp.	K	1	NCTC 10232	L-Lys-L-Ala$_2$(L-Ser)	19b	Hladny and Kandler
S. salivarius I	K	1	Kiel 21372	L-Lys-L-Ala$_{2-3}$	19a	Hladny and Kandler
S. salivarius II		2	Kiel 21367	L-Lys-L-Thr-Gly	20b	141a
Streptococcus sp.	L	3	Kiel 8898, 13009, 13026	L-Lys-L-Ala$_{2-3}$	19a	Hladny and Kandler
Streptococcus sp.	L	1	NCTC 3166	L-Lys-L-Ala-Gly	19d	Hladny and Kandler
S. lactis	M	2	Kiel 25791, 25792	L-Lys-L-Ala$_{2-3}$	19a	Hladny and Kandler
Streptococcus sp.	N	7	ATCC 7962	L-Lys-D-Asp	9	Hladny and Kandler, 337
Streptococcus sp.	O	2	NCTC 6119, 8029	L-Lys-direct	18	Hladny and Kandler
Streptococcus sp.	P	1	Kiel 21361	L-Lys-L-Ala$_{3-4}$	13a	Hladny and Kandler
Streptococcus sp.	P	1	NCTC 9824	L-Lys-L-Ala$_{1-2}$	19a	Hladny and Kandler
Streptococcus sp.	Q	2		L-Lys-D-Asp	9	358, Hladny and Kandler
Streptococcus sp.	R	1	NCTC 10234	L-Lys-direct	18	Hladny and Kandler
Streptococcus sp.	U	1	Kiel 7185	L-Lys-Ala$_{2-3}$	19a	Hladny and Kandler
S. milleri		1	Kiel 20205	L-Lys-L-Thr-L-Ala(L-Ser)	20a	141a
S. milleri		2		L-Lys-L-Thr-Gly	20b	141a
S. mutans		2	Kiel 25859, 25861	L-Lys-L-Thr-L-Ala	20a	141a

(*Table 20 continued*)

TABLE 20. *Continued*

Species	Serological group	No. of strains	Culture collection[a] strain no.	Type of peptidoglycan	Fig.	Reference
Streptococcus sp.		1		L-Lys-L-Ala_2-Gly-L-Ala	19e	Hladny and Kandler
S. thermophilus		4		L-Lys-L-Ala_{2-3}	19a	336
S. viridans I; II; IV		3	Kiel 104; 25836; 25818	L-Lys-direct	18	Hladny and Kandler
S. viridans IV		1	Kiel 7733	L-Lys-L-Ala-Gly	19d	Hladny and Kandler

[a] Only those numbers given by culture collections are mentioned. The other strains are own isolates or were obtained from private collections and can be found in the corresponding references.

[b] J. Hladny, and O. Kandler. Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. I Orig., *in press*.

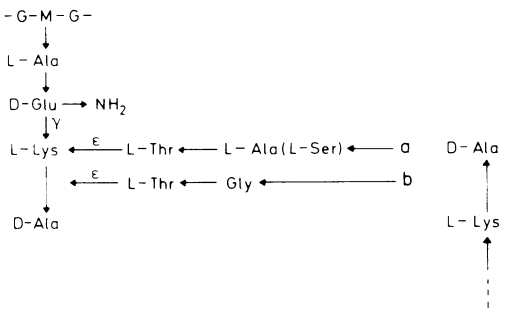

FIG. 19. *Fragments of the primary structures of threonine-containing peptidoglycans of streptococci as listed in Tables 20 and 22. (a) L-Lys-L-Thr-L-Ala(L-Ser); (b) L-Lys-L-Thr-Gly.*

Streptococcus sp. (group U). The peptidoglycan type L-Lys-L-Ala-Gly was found among streptococci in one strain of group L and in *S. viridans* IV only. These two strains reveal identical physiological properties, and it is suggested that they are members of the same species (Hladny, Ph.D. thesis, Technical University, Munich, 1971).

The strains containing Thr residues in their peptidoglycan also show several similar physiological activities, especially *S. bovis*, *S. milleri*, and *S. equinus*. Only *S. salivarius* II and *S. mutans* differ, since they do not grow at 45 C, show less resistance against bile, and do not ferment mannitol and sorbitol.

Among the strains containing isoasparagine in their peptidoglycan (Fig. 9; L-Lys-D-Asp type), one may distinguish two groups of more closely related strains. The first group includes *S. faecium* and *S. avium* (group Q) which are quite similar in their physiological properties. The other group consists of *S. lactis* and *S. cremoris*, organisms which predominantly occur in milk or dairy products and which belong to serological group N.

The directly cross-linked, L-Lys-containing peptidoglycan is a rather rare type. It has only been found in aerococci and some streptococci up to now. Among the streptococci containing this peptidoglycan type, there are two groups of more closely related strains. Strains of *Streptococcus* sp. (group R) and *S. viridans* I show a broad spectrum of physiological activities and contain rhamnose and little phosphorus in their cell walls. Strains of groups K and O, *S. sanguis* II, and *S. viridans* II and IV, on the other hand, show fewer physiological activities and contain no rhamnose but much more phosphorus in their cell walls. A critical consideration of the variation of the peptidoglycan within the genus *Streptococcus* will certainly be very helpful for a more rational subdivision of this genus into species.

Genus Peptostreptococcus. The genus *Peptostreptococcus* comprises anaerobic streptococci, which may be pathogenic. Some species are found as part of the normal bacterial flora of respiratory and gastrointestinal tracts (49). The classification of this group of bacteria is considered to be unsatisfactory, because the data on growth conditions and biochemical activities are scarce. Little is also known about the chemical composition of their cell walls. Bahn et al. (30) have studied the chemical composition of the cell walls of two strains of *P. putridus*, two strains of *P. intermedius*, and one strain of *P. elsdenii*. The strain of *P. elsdenii* did not belong to the genus *Peptostreptococcus* and was found to be a gram-negative organism. The molar ratios of the main amino acids of the cell walls of the other two species are listed in Table 23. From these ratios it is suggested that *P. putridus* contains a Lys-D-Asp peptidoglycan type and the *P. intermedius* strains, a Lys-L-Ala_3 type. A strain similar in its physiological behavior to *P. evolutus* was isolated from feces and its peptidoglycan type established (339; Table 23). The cross-linkage of the peptide subunits is performed by a Gly-L-Ala

TABLE 21. *Distinction of strains of Streptococcus sp. (serological group K) and strains of S. salivarius*

Species	Strain no.	Serological group	Peptidoglycan type	Formation of levan
Streptococcus salivarius I	Kiel 21372	K	L-Lys-L-Ala_{2-3}	+
S. salivarius II	Kiel 21367		L-Lys-L-Thr-Gly	+
Streptococcus sp.	Kiel 226, 13272	K	L-Lys-direct	−
Streptococcus sp.	NCTC 10232	K	L-Lys-L-Ala(L-Ser)-L-Ala	−

TABLE 22. *Grouping of strains of streptococci according to their peptidoglycan types*

Peptidoglycan type	Species or serological groups
L-Lys-L-Ala_{1-2}	*Streptococcus* sp. (groups F, G, and P)
L-Lys-L-Ala_{2-3}	*S. pyogenes* (group A), *S. equisimilis*, *S. zooepidemicus*, *S. equi*, *S. dysgalactiae* (all group C), *S. faecalis* (group D), *S. sanguis* (group H), *S. salivarius* (group K), *Streptococcus* sp. (groups F, L. M and U), *S. thermophilus*
L-Lys-L-Ala_{3-4}	*S. uberis* (group E), *Streptococcus* sp. (group P)
L-Lys-L-Ala-L-Ala(L-Ser)	*S. agalactiae* (group B), *Streptococcus* sp. (group K)
L-Lys-L-Ala_2-L-Ala(L-Ser)	*Streptococcus* sp. (group G)
L-Lys-L-Ala-Gly	*Streptococcus* sp. (group L), *S. viridans* IV
L-Lys-L-Thr-L-Ala	*S. equinus* (group D), *S. mutans*
L-Lys-L-Thr-L-Ala(L-Ser)	*S. bovis*, *S. equinus* (both group D), *S. milleri*
L-Lys-L-Thr-Gly	*S. equinus* (group D), *S. salivarius* II
L-Lys-D-Asp	*S. faecium*, *S. faecium* var. *durans* (both group D), *S. avium* (group Q); *S. lactis* and *S. cremoris* (both group N)
L-Lys-direct	*Streptococcus* sp. (group R), *S. viridans* I, *S.* sp. (groups K and O), *S. sanguis* II, *S. viridans* II and IV

TABLE 23. *Amino acid composition of the cell walls of Peptostreptococcus*

Species	Molar ratio of amino acids					Suggested peptidoglycan	Fig.	Reference
	Glu	Lys	Ala	Asp	Gly			
P. putridus	1.0	0.8	1.6	0.8		L-Lys-D-Asp	9	30
P. intermedius	1.0	1.1	4.0			L-Lys-L-Ala_2	19a	30
P. evolutus	1.0	1.1	2.8		1.0	L-Lys-L-Ala-Gly	19d	339

peptide (Fig. 20) as in some streptococci (vide supra). These few data indicate a diversity of peptidoglycan types similar to that found within the genus *Streptococcus*.

Genus Lactobacillus. The genus *Lactobacillus* is divided into four subgenera by using the grouping of Orla-Jensen (277) but using his genera as subgenera.

(i) All the strains of the subgenus *Thermobacterium* contain the Lys-D-Asp type of peptidoglycan (Table 24, Fig. 9) as described by Kandler (168, 169). The structure of the cell wall of one strain of *L. acidophilus* has been studied in greater detail (65). Besides peptidoglycan, a neutral and an anionic polysaccharide are the main structural components of these cell walls. The peptide subunits of the peptidoglycan are cross-linked, as in the other thermobacteria, by single D-isoasparaginyl residues. All the muramic acid residues are peptide substituted, and about 60 to 70% of them carry O-acetyl substituents on C^6. The neutral polysaccharide is composed of equimolar amounts of glucose, galactose, and rhamnose and is linked by phosphodiester groups to muramic acid residues. The anionic polysaccharide is a glycerophosphate-polyglucose. The backbone is composed of a (α or β) 1,6-linked polyglucose polymer. Monomeric α-glycerol phosphate side chains are attached to the glucose residues on C^2 or C^4 position.

(ii) The subgenus *Streptobacterium* can be subdivided into two groups based on the occurrence of the L-Lys-D-Asp and the direct cross-linked m-Dpm type as shown in Table 25. The latter type was found in *L. plantarum* and *L. inulinus* and was described in detail by Weiss et al. (406) and by Matsuda et al. (241, 242). With

the exception of *L. plantarum* var. *mobilis*, all strains of *L. plantarum* contain teichoic acid. In one case the structure of the teichoic acid (ribitol teichoic acid) has been elucidated (12).

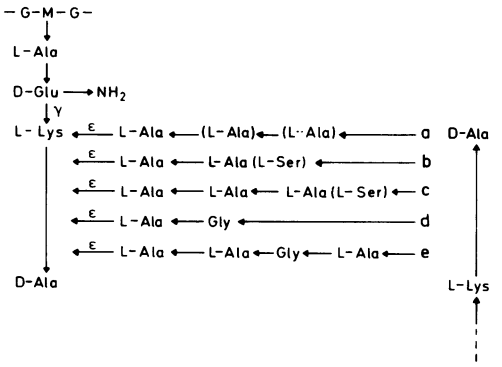

FIG. 20. *Fragments of the primary structures of peptidoglycans of streptococci as listed in Tables 20 and 22. Amino acids in parentheses may be absent or may partially replace the corresponding amino acid. (a) L-Lys-L-Ala$_{1-3}$; (b) L-Lys-L-Ala-L-Ala(L-Ser); (c) L-Lys-L-Ala$_2$-L-Ala(L-Ser); (d) L-Lys-L-Ala-Gly; (e) L-Lys-L-Ala$_2$-Gly-L-Ala.*

The L-Lys-D-Asp type of peptidoglycan of *L. coryneformis* was studied in detail by Plapp and Kandler (303) and that of *L. casei* by Hungerer et al. (151). In cell walls of *L. casei* ssp. *tolerans*, a glycerol-containing teichoic acid was found in large amounts.

The exclusion of *L. plantarum* from the

TABLE 24. *Species and strains of the subgenus Thermobacterium studied for their peptidoglycan type*[a]

Species	No. of strains	ATCC[b] no.
Lactobacillus bulgaricus	6	11842
L. helveticus	4	15009
L. jugurti	3	521
L. lactis	35	12315
L. acidophilus	35	4356
L. salivarius	2	11741
L. delbrückii	1	11978
L. leichmannii	1	4797
L. jensenii	1	25258

[a] All contain the L-Lys-D-Asp type (variation A4α, Fig. 9).

[b] See footnote *a* in Table 20.

TABLE 25. *Distribution of peptidoglycan types within the subgenera Streptobacterium and Sporolactobacillus (variations A1α and A1γ)*

Species	No. of strains	ATCC[a] no.	Type	Fig.	Reference
Lactobacillus casei ssp. *casei*	10	393	L-Lys-D-Asp	9	168, 169
L. casei					
ssp. *pseudoplantarum*[b]	5		L-Lys-D-Asp	9	168
ssp. *rhamnosus*	2	7469	L-Lys-D-Asp	9	168
ssp. *tolerans*[b]	2		L-Lys-D-Asp	9	168
ssp. *fusiformis*[c]	3		L-Lys-D-Asp	9	169
L. sake	1	15521	L-Lys-D-Asp	9	168, 169
L. coryneformis					
ssp. *coryneformis*[b]	4		L-Lys-D-Asp	9	168, 169, 303, 304
ssp. *torquens*[b]	2		L-Lys-D-Asp	9	168, 169
L. curvatus[b]	4		L-Lys-D-Asp	9	168, 169
L. xylosus	1	15577	L-Lys-D-Asp	9	Kandler, *unpublished data*
L. zeae	1	15820	L-Lys-D-Asp	9	Kandler, *unpublished data*
L. plantarum					
ssp. *plantarum*	80	8014	m-Dpm-direct	6	168, 169, 406
ssp. *arabinosus*	40	10012	m-Dpm-direct	6	168, 169, 406
ssp. *rudensis*	1	13649	m-Dpm-direct	6	Kandler, *unpublished data*
var. *mobilis*[d]	3		m-Dpm-direct	6	168, 169, 406
L. inulinus	1	15538	m-Dpm-direct	6	168, 169, 406

[a] See footnote *a* in Table 20.

[b] Isolated and described by Abo-Elnaga and Kandler (1).

[c] Isolated and described by Eschenbecher (100).

[d] Isolated and described by Harrison and Hansen (135).

subgenus *Streptobacterium* was proposed by Kandler (168, 169). It seems feasible to put it with *L. inulinus* in a separate subgenus *Sporolactobacillus* (194) which is characterized by showing some properties of the genus *Bacillus*, i.e., type of peptidoglycan and teichoic acid, and in some cases nitrate reduction, motility, and sporulation. This subgenus would be distinguished from the genus *Bacillus* by the absence of cytochromes, and these organisms are therefore not able to carry out a true oxidative metabolism.

(iii) The types of peptidoglycans found in species of the subgenus *Betabacterium* are listed in Table 26. The predominant type is again the Lys-D-Asp type, but especially the recently described species show considerable variations in the interpeptide bridge. In the peptidoglycans of *L. fermentum* and *L. cellobiosus*, L-Lys residues are replaced by L-Orn residues (301, 302, 422). Both species possess identical GC content and electrophoretically identical lactic acid dehydrogenases (103). It seems feasible that they should be united in one species. They may be distinguished as subspecies on the basis of the fermentation pattern. Table 26 contains some other strains called *L. fermentum* which contain L-Lys in their peptidoglycan. As shown in our laboratory these strains do not belong to *L. fermentum*. Strain ATCC 9338 is identical with *L. brevis* according to the spectrum of fermented sugars and the electrophoretic mobility of the lactic acid dehydrogenase. (In an earlier publication [169] it was reported that *L. fermentum* ATCC 14931 contained a peptidoglycan of L-Lys-D-Asp type. This was due to a clerical error; the correct number of the strain studied is ATCC 9338. The other five strains mentioned in the same publication were similar to ATCC 23272 were isolated from manure. Unfortunately, none of the genuine *L. fermentum* strains investigated by Williams and Sadler [422] was included in these studies.)

Strain *L. fermentum* ATCC 23272 (Reuter type II) differs from strain ATCC 14931 (type strain) as well as from *L. brevis* with respect to the GC content, sugar fermentation, and electrophoretic mobility of the lactic acid dehydrogenases. It should be considered as a separate species, not yet described.

The recently described species *L. viridescens* (170, 174, 273) and *L. coprophilus* (144, 169, 304) show different peptidoglycan types. The interpeptide bridges do not consist of D-isoasparaginyl residues but of L-Ala-L-Ala-L-Ser, L-Ser-

TABLE 26. *Distribution of peptidoglycan types within the subgenus Betabacterium*

Species	No. of strains	ATCC[a] no.	Type	Fig.	Reference
Lactobacillus fermentum ...	1	23272	L-Lys-D-Asp	9	Kandler, *unpublished data*
L. fermentum	1	9338	L-Lys-D-Asp	9	168, 169
L. cellobiosus	4	11739	L-Orn-D-Asp	9, with Orn instead of L-Lys	169, 303
L. fermentum	1	11740	L-Orn-D-Asp	9, with Orn	422
L. fermentum	1	14931	L-Orn-D-Asp	instead of L-Lys	422
L. fermentum	1	14932	L-Orn-D-Asp	9, with Orn instead of L-Lys	422
L. brevis	10	14869	L-Lys-D-Asp	9	168, 169
L. buchneri	1	4005	L-Lys-D-Asp	9	168, 169
L. fructovorans	1	8288	L-Lys-D-Asp	9	168, 169
L. malefermentans	1	11306	L-Lys-D-Asp	9	168, 169
L. pastorianus	1	8291	L-Lys-D-Asp	9	168, 169
L. parvus	1	11305	L-Lys-D-Asp	9	168, 169
L. frigidus	1	11307	L-Lys-D-Asp	9	168, 169
L. hilgardii	1	8290	L-Lys-D-Asp	9	168, 169
L. viridescens					
ssp. *viridescens*	6	12706	L-Lys-L-Ala-L-Ser	21a	169, 174
ssp. *minor*	4		L-Lys-L-Ser-L-Ala_2	21b	168, 169
L. coprophilus					
ssp. *coprophilus*	3		L-Lys-L-Ala_2	21c	144, 169
ssp. *confusus*	14	10881	L-Lys-Ala	21c	168, 169

[a] See footnote *a* in Table 20.

L-Ala, L-Ala-L-Ala, or L-Ala. The amino acid compositions of the interpeptide bridges of the two subspecies of *L. viridescens* (170) are quite similar but the amino acid sequences are reversed (Fig. 21a and b). Thus the differences in the peptidoglycan structure confirm the separation of these two subspecies which was originally based on physiological and morphological evidence.

The two subspecies of *L. coprophilus* are distinguished by the number of alanine residues in the interpeptide bridge and by the ability of one to form dextran (144). Although the peptidoglycan type is quite similar to that of *Leuconostoc* (vide infra), both subspecies belong to the subgenus *Betabacterium* since they produce D,L-lactic acid and split arginine.

L. coprophilus and *L. viridescens* form, in contrast to other species of the genus *Betabacterium*, irregular rods, and in particular both subspecies of *L. coprophilus*, and *L. viridescens* ssp. *minor* can easily be confused with *Leuconostoc*. They may constitute an intermediary group between the genera *Leuconostoc* and *Betabacterium*. Williams and Sadler (422) report the occurrence of Gly in cell walls of some strains of heterofermentative lactobacilli. These cell walls were reinvestigated in our laboratory by quantitative amino acid analysis and by determining the peptide pattern of partial acid hydrolysates. It could be shown that these cell walls were contaminated by protein. The real amino acid composition of the peptidoglycan of the strains mentioned by Williams and Sadler (422) is in agreement with the data given in Table 26.

Genus Leuconostoc. The genus *Leuconostoc* was classified according to Garvie (104, 106) and Whittenbury (417). The peptidoglycan types of the different species are listed in Table 27.

The majority of the strains contain a peptidoglycan of the Lys-L-Ser-L-Ala type. The remainder of the strains reveal di-L-Ala, L-Ser-Ala, or L-Ser-L-Ser peptides as interpeptide bridges. The correlation of the peptidoglycan type with the present grouping of the strains

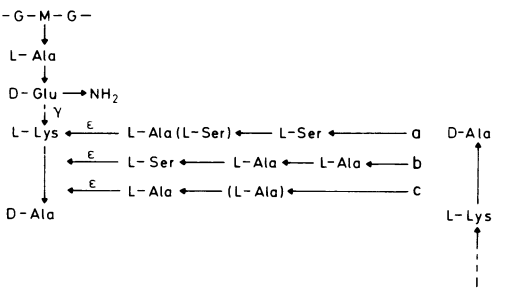

FIG. 21. *Fragments of the primary structures of peptidoglycans of lactobacilli and leuconostocs. (a) L-Lys-L-Ala(L-Ser)-L-Ser; (b) L-Lys-L-Ser-L-Ala$_2$; (c) L-Lys-L-Ala$_{(2)}$.*

TABLE 27. *Distribution of peptidoglycan types within the genus Leuconostoc*

Species	No. of strains	ATCC or NCDO[a] no.	Type	Fig.	Reference
Leuconostoc cremoris	8	NCDO 828	L-Lys-L-Ser-L-Ala$_2$	21b	169
L. lactis	8	NCDO 533, 546	L-Lys-L-Ser-L-Ala$_2$ plus L-Lys-L-Ala$_2$	21b 21c	169
L. paramesenteroides ssp. ssp. *paramesenteroides*	2	NCDO 803, 883	L-Lys-L-Ala$_2$	21c	169
ssp. *lactophilum*	5		L-Lys-L-Ser-L-Ala$_2$	21b	169
L. gracile (syn. *L. oenos*) .	23	NCDO 1668, 1669, 1674, 1675, 1694, 1696, 1705, 1707, 1823, 1890, 1893	L-Lys-L-Ala-L-Ser plus L-Lys-L-Ser-L-Ser	21a 21a	169
L. dextranicum	1	NCDO 517	L-Lys-L-Ser-L-Ala$_2$	21b	169
L. mesenteroides ssp. *mesenteroides* ..	18	NCDO 183, 523, 538; ATCC 8082, 8086, 8293, 8357, 8359, 9135, 10830, 10830a, 10878, 12291, 14430, 14935	L-Lys-L-Ser-L-Ala$_2$	21b	169, 174
ssp. *amelibiosus*	4	ATCC 13146, 10882, 10883, 11449	L-Lys-L-Ala$_2$	21c	169

[a] See footnote *a* in Table 20.

into species was only perfect in the case of *L. cremoris*. Each of the other species show two different types of peptidoglycan. Strains of both species *L. paramesenteroides* and *L. mesenteroides*, which differ in their peptidoglycan, can also be distinguished by their fermentation pattern; therefore new subspecies of these species were proposed (Holzapfel, Ph.D. thesis, Technical University, Munich 1969).

L. gracile (Syn. *L. oenos*, Garvie, 105) is an unusual species, only isolated from wine. It is characterized by its ability to grow at low *p*H and to form peroxide and by its inability to produce dextran. All strains investigated so far belong to the peptidoglycan types Lys-L-Ala-L-Ser or Lys-L-Ser_2. In most of the strains the majority of the interpeptide bridges are formed by L-Ser-L-Ala and the minority by L-Ser-L-Ser. Only in a few strains is the L-Ser_2 interpeptide bridge the predominant one. The ratio of both types of interpeptide bridges is genetically determined since a variation of the Ser or Ala content of the growth medium did not change the ratio of the two interpeptide bridges significantly (169).

Genus Pediococcus. All the strains of the genus *Pediococcus* (*P. cerevisiae*, *P. acidolactici*, *P. pentosaceus*) contain the Lys-D-Asp peptidoglycan type (169, 416). The occurrence of aspartic acid in the cell wall hydrolysate is an important criterion to separate *Pediococcus* from *Leuconostoc* and *Aerococcus*, since no strain of the latter genera contains Asp in its peptidoglycan.

Genus Bifidobacterium. The genus *Bifidobacterium* is distinguished by its characteristic fermentation pattern. Only those anaerobic strains forming acetic acid and lactic acid (about 3:2), but no CO_2, are considered to be bifidobacteria (90, 328). For the grouping of the strains into species, the classification schemes of Reuter (314) and Scardovi et al. (331) were used (Table 28). There is a rather good correlation between the type of peptidoglycan and the defined species (169, Table 28). *B. bifidum* contains the peptidoglycan type. L-Orn-D-Ser-D-Asp as described in detail by Koch et al. (199) and Veerkamp (395). The Lys-Gly type was first described for *Bacillus constellatus* (176). This organism is a *Bifidobacterium*, and such strains are now classified as *B. asteroides* (329). The same peptidoglycan type was also found in *B. infantes*, *B. breve*, and *B. parvulorum*.

A very complex interpeptide bridge (L-Orn-

TABLE 28. *Distribution of peptidoglycan types within the genus Bifidobacterium*

Species	No. of strains	ATCC no.[a]	Type	Variation	Fig.	Reference
B. infantis	6	15697	L-Lys-Gly	A3α	23	169
B. breve	4	15700, 15701	L-Lys-Gly	A3α	23	169
B. asteroides[b]	1		L-Lys-Gly	A3α	22	169, 176
B. globosum[b]	6		L-Orn(Lys)-L-Ala_3	A3β	22c	169
B. parvulorum	1	15698	L-Lys-Gly	A3α	23	Kandler, *unpublished data*
B. lactentis	1		L-Orn-L-Ser-L-Ala-L-Thr-L-Ala	A3β	22b	169, 200
B. longum	20	15707, 15708	L-Orn-L-Ser-L-Ala-L-Thr-L-Ala	A3β	22b	169, 200
B. suis[b]	1		L-Orn-L-Ser-L-Ala-L-Thr-L-Ala	A3β	22b	169
B. ruminale[b]	4		L-Lys-D-Glu	A4α	17a	Lauer and Kandler, *unpublished data*
B. thermophilum	4	25525	L-Lys-D-Glu	A4α	17a	Lauer and Kandler, *unpublished data*
B. indicum[b]	1		L-Lys-D-Asp	A4α	9	Lauer and Kandler, *unpublished data*
B. coryneforme[b]	1		L-Lys-D-Asp	A4α	9	Lauer and Kandler, *unpublished data*
B. eriksonii	1	15423	L-Lys-D-Asp	A4α	9	Lauer and Kandler, *unpublished data*
B. bifidum	9	11863	L-Orn-D-Ser-D-Asp	A4β	22a	169, 199
B. adolescentis	15	15704/05/06	L-Orn(Lys)-D-Asp	A4β	9	169
	2		L-Lys-L-Ser-L-Ala_2	A3α	21b	169

[a] See footnote *a* in Table 20.
[b] Obtained from Dr. Scardovi, Piacenza, Italy.

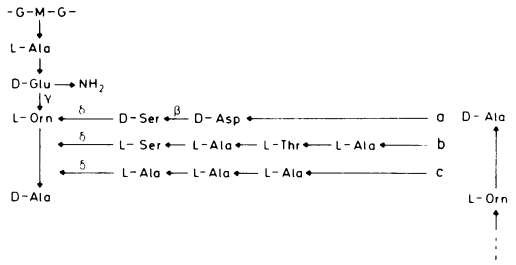

FIG. 22. *Fragments of the primary structures of ornithine-containing peptidoglycans of bifidobacteria (A3β and A4β). (a) L-Orn-D-Ser-D-Asp; (b) L-Orn-L-Ser-L-Ala-L-Thr-L-Ala; (c) L-Orn-L-Ala*$_3$.

L-Ser-L-Ala-L-Thr-L-Ala) is typical for the peptidoglycan of *B. longum* (Fig. 22b, 200). It was also detected in one strain of *B. lactentis* and *B. suis*.

Strains of *B. adolescentis* contain either the Lys-L-Ser-L-Ala$_2$ type which is found in many strains of *Leuconostoc* or the L-Orn-(L-Lys)-D-Asp type which is typical for most of the lactobacilli. The latter type, however, differs from that of lactobacilli, since L-Orn and L-Lys are both present in varying amounts in the same peptidoglycan. The ratio of Orn/Lys varies with different strains of *B. adolescentis*, but is not influenced by exogenous addition of Lys or Orn.

A similarly varying ratio of Lys and Orn was found in strains isolated from the rumen of the sheep (145). These strains were recently described as a new species *B. globosum* (330). The interpeptide bridge here consists of a tri-L-alanine peptide (Fig. 22c).

B. indicum and *B. coryneforme* contain the L-Lys-D-Asp type as do many strains of lactobacilli and some streptococci. The peptide subunits of the peptidoglycans of *B. ruminale* and *B. thermophilum* are also cross-linked by a D-dicarboxylic amino acid, namely D-Glu as in the genus *Planococcus* (340, Fig. 18a). These two species are most likely identical.

Family Bacillaceae. This group of spore-forming, gram-positive rods is subdivided into two genera: the aerobic or microaerophilic strains are included in the genus *Bacillus*, the anaerobic organisms in the genus *Clostridium*.

Genus Bacillus. The vast majority of the strains of the genus *Bacillus* belong to the same peptidoglycan type, namely the directly cross-linked m-Dpm type (A1γ, Fig. 6). The species showing this type are listed in Table 29. The peptidoglycan of a few of these species have been studied in greater detail (Fig. 23). The exact structure of *B. megaterium* KM was established by Bricas et al. (50), that of *B. licheniformis* by Hughes (146, 147), and that of *B. subtilis* by Warth and Strominger (402). H. Ranftl in our laboratory (H. Ranftl, Ph.D. thesis, Technical University, Munich, 1972) has also studied in detail a large number of cell walls of various bacilli. Despite the similarity in the primary structure of their peptidoglycan, there are differences in the amidation of carboxyl groups, the degree of cross-linkage, the chain length of peptide subunits, and the occurrence of D,D-Dpm besides m-Dpm. Most of the bacilli contain only one amide group in the peptidoglycan; either the α-carboxyl group of D-Glu can be amidated as in the case of *B. licheniformis* (254) or the carboxyl group of m-Dpm which is not involved in a peptide bond is amidated as in the case of *B. subtilis* (402). Some bacilli have no amide group at all, e.g., *B. megaterium* (50), *B. lentus*, and *B. firmus* (H. Ranftl, Ph.D. thesis, Technical University, Munich, 1972). In the peptidoglycan of *B. megaterium*, besides m-Dpm residues, D,D-Dpm residues were also found (50). The D,D-Dpm residues seemed to be involved in another mode of cross-linkage (370). The D,D-Dpm residues may replace a D-Ala residue by a transpeptidation reaction at the C terminus of a peptide subunit and can connect with an adjacent peptide subunit so that a cross-linkage is formed between an amino group of D,D-Dpm and a carboxyl group of a D-Ala residue.

The peptide subunits usually consist of tetra- or tripeptides and, sometimes pentapeptides can also occur. The ratio of tetra- to tripeptides can vary from strain to strain. A high content of tripeptides is paralleled by a low degree of cross-linkage. These different variations of the directly cross-linked, m-Dpm-containing peptidoglycan may be characteristic for the various species, but it is still too early for a taxonomic evaluation. Another helpful feature for the grouping of bacilli may be the composition of the polysaccharide and teichoic acid polymers of the cell walls. Very often glycerol-containing teichoic acids are found in cell walls of bacilli (H. Ranftl, Ph.D. thesis, Technical University, Munich, 1972), whereas ribitol teichoic acids are rather uncommon and have been detected as yet only in *B. subtilis* (20, 21). Teichuronic acid was found in *B. licheniformis* (148). Besides these anionic polymers, neutral polysaccharides also occur which can be composed of amino sugars such as glucosamine, galactosamine, or mannosamine, or all three, and other sugars such as glucose, galactose, or mannose, or all three.

A few unique bacilli characterized by the production of spherical endospores have a different type of peptidoglycan. *B. sphaericus*

TABLE 29. *Distribution of directly cross-linked, meso-Dpm containing peptidoglycan (A1α) in the genus Bacillus*[a]

Species	No. of strains	Culture collections strain no.[b]	Reference
Bacillus alvei	4	ATCC 6344, 6348, 6349, 10871	Ranftl[c]
B. aminovorans	1	ATCC 7046	Ranftl
B. aneurinolyticus	1	ATCC 12856	Ranftl
B. anthracis	1	Fort Detrick V_1B-189	312
B. aporrheus	1	ATCC 9500	Ranftl
B. badius	1	ATCC 14574	Ranftl
B. brevis	1	ATCC 8246	Ranftl
B. cereus	6	ATCC 11778, 13824, 14579	Ranftl, 9, 327
B. cereus ssp. *mycoides*	2	ATCC 6462	Ranftl
B. circulans	1	ATCC 9966	Ranftl
B. coagulans	3	ATCC 7050, 10545	Ranftl, 101a
B. firmus	1	ATCC 8247	Ranftl
B. freudenreichii	1	ATCC 7053	Ranftl
B. lactimorbus	1	ATCC 246	Ranftl
B. laterosporus	2	ATCC 64	Ranftl
B. lentimorbus	1	ATCC 14707	Ranftl
B. lentus	1		Ranftl
B. licheniformis	3	ATCC 14580, NCTC 6346	146, 147
B. macerans	2	ATCC 843, 8244	Ranftl
B. megaterium	2	ATCC 10778	Ranftl, 50, 327
B. natto	1	ATCC 15245	Ranftl
B. palustris	1	ATCC 15518	Ranftl
B. pantothenticus	1	ATCC 14576	Ranftl
B. polymyxa	1	ATCC 10401	Ranftl
B. pulvifaciens	1	ATCC 13537	Ranftl
B. pumilis	3	ATCC 7061, NCTC 6353	Ranftl, 327
B. serositidis	1	ATCC 7063	Ranftl
B. stearothermophilus	5	ATCC 7953	Ranftl, 101a, 327, 374
B. subtilis	6	ATCC 6051	Ranftl, 327, 402, 430, 431
B. subtilis var. *aterrimus*	1	ATCC 7060	Ranftl
B. subtilis var. *niger*	1	ATCC 9372	Ranftl
B. thiaminolyticus	1	ATCC 11376	Ranftl
B. thuringiensis	3	ATCC 10792	Ranftl, 193

[a] For primary structure see Fig. 6.
[b] See footnote *a* in Table 20.
[c] H. Ranftl, Ph.D. thesis, Technical University, Munich, 1972.

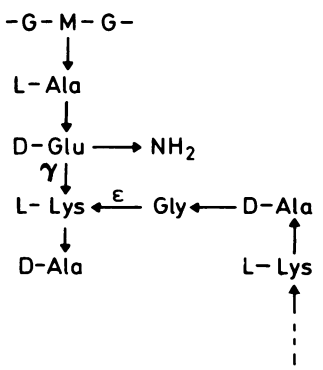

FIG. 23. *Fragment of the primary structure of peptidoglycans of B. infantis, B. breve, and B. asteroides and some coryneform organisms (A3α). L-Lys-Gly.*

contains m-Dpm in its spores but its vegetative cell walls contain Lys and Asp (306). The primary structure of the vegetative peptidoglycan is very similar to that of lactobacilli (Fig. 9). It differs only in the absence of C-terminal D-alanine residues and of amidated α-carboxyl groups of D-Glu (150).

B. pasteurii is distinguished from other bacilli not only by the production of spherical endospores but also by its ability to metabolize urea, a physiological property which it has in common with *Sporosarcina ureae*. As mentioned above, the peptidoglycan types of these organisms are also quite similar.

To date the peptidoglycans of three strains of *B. pasteurii* have been studied in detail (H. Ranftl and O. Kandler, Z. Naturforsch., *in press*). The structure of strain ATCC 6453 is

depicted in Fig. 24b. The interpeptide bridge consists of β-D-Asp-L-Ala.

Strain ATCC 11859, called *B. pasteurii*, shows the same peptidoglycan type as *B. sphaericus* and is most likely a urease-producing *B. sphaericus*. A third strain (own isolate) contains a β-D-Asp-L-Ser interpeptide bridge. Some of the L-Ser residues are replaced by L-Ala. Although more strains of both species need to be investigated, the peptidoglycan type is most likely a good criterion to distinguish *B. pasteurii* from *B. sphaericus*.

Genus Clostridium. The cell wall composition of these anaerobic spore-forming organisms of the genus *Clostridium* was studied primarily by Cummins and co-workers (61, 73, 77). They determined qualitatively the sugar and amino acid composition of the cell walls of various clostridia. There are also a few studies on the quantitative amino acid composition (221, 296, 376). All the available data on the amino acid composition of the cell walls of clostridia are compiled in Table 30.

Most of the species investigated contain only m-Dpm, Ala, and Glu. Unpublished studies from our laboratory on cell walls of *C. butyricum* have shown that the peptidoglycan of this organism belongs to the directly cross-linked, m-Dpm-containing type (A1γ). The other m-Dpm-containing peptidoglycans of the genus *Clostridium* presumably show the same type.

Four different species reveal (instead of m-Dpm) L,L-Dpm and, in addition, Gly in their cell walls. The primary structure of the peptidoglycan of *C. perfringens* was established by Leyh-Bouille et al. (221). This species shows the same peptidoglycan type as that described before for *Propionibacterium petersonii* and some *Streptomyces* sp. (Fig. 15a). The three other species *C. fallax*, *C. pectinovorum*, and *C. pseudofallax* probably belong to the same type.

The species *C. innocuum*, *C. paraputrificum*, and *C. tertium* contain L-Lys instead of Dpm in their vegetative cell walls. Since no detailed studies are available, the peptidoglycan type of these species is unknown. *C. innocuum* has probably been misclassified in the genus *Clostridium*, since it differs not only in the amino acid composition of its cell walls but also in its GC content (43%) from the other strains of clostridia (GC content, 22–28%) (77).

The status of the other clostridia which contain no Dpm is uncertain despite their rather low GC content (24–27%) which is in good agreement with that of the other members of the genus clostridia. Further studies are necessary to clarify the taxonomic position of these organisms.

The peptidoglycans within the genus *Clostridium*, as in the genus *Bacillus*, are rather uniform. Most of the clostridia contain the directly cross-linked m-Dpm peptidoglycan type (variation A1γ), but a few species are distinguished by the occurrence of the L,L-Dpm-Gly type. The occurrence of other peptidoglycan types in strains called *Clostridium* can be taken as an indication that these organisms may be improperly classified.

The determination of the structure of the polysaccharides of the cell walls of clostridia may be a useful tool for characterization of species and infraspecific taxa within the genus. Cummins and Johnson (77) had already achieved some separation by means of a qualitative determination of the sugar composition in the cell walls.

Family Corynebacteriaceae. The family *Corynebacteriaceae* is characterized by irregular rod-shaped cells which usually occur in angular or palisade formation due to snapping division. Moreover, a marked diversity in morphology and a vegetative life cycle are often found. According to the 7th edition of *Bergey's Manual* (49), the family *Corynebacteriaceae* comprises six different genera: *Corynebacterium*, *Arthrobacter*, *Microbacterium*, *Cellulomonas*, *Listeria*, and *Erysipelothrix*. More recent studies have shown that almost all of the organisms included in the family *Brevibacteriaceae* are also typical coryneform bacteria (161, 264, 332; Cziharz, Diplom Thesis, Technical University, Munich, 1969). Therefore, the organisms classified in the family *Brevibacteriaceae* (genera *Brevibacterium* and *Kurthia*) will be discussed together with the *Corynebacteriaceae*.

The lack of proper criteria for differentiation makes the classification of most of the coryneform organisms rather unsatisfactory. In the

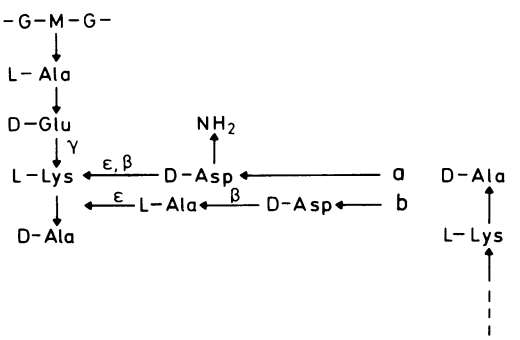

FIG. 24. *Fragments of the primary structures of uncommon peptidoglycans in the genus Bacillus. (a) L-Lys-D-Asp, B. sphaericus; (b) L-Lys-L-Ala-D-Asp, B. pasteurii.*

TABLE 30. *Amino acid composition of cell walls of the genus Clostridium*

Species	No. of strains	m-Dpm	Glu	Ala	L, L-Dpm	Gly	Lys	Reference
C. acetobutylicum	4	+	+	+	–	–	–	77
C. amylolyticum	1	+	+	+	–	–	–	73
C. aurantibutyricum	2	+	+	+	–	–	–	77
C. beijerinckii	2	+	+	+	–	–	–	77
C. bifermentans	16	+	+	+	–	–	–	274
C. botulinum	22	+	+	+	–	–	–	73
C. botulinum	1	0.85	1.0	1.85	–	–	–	376
C. butyricum	30	+	+	+	–	–	–	73, 77, 322
C. fallax	3	+	+	+	–	–	–	77
C. felsineum	2	+	+	+	–	–	–	77
C. haemolyticum	2	+	+	+	–	–	–	77
C. histolyticum	2	+	+	+	–	–	–	322, 380
C. lacto-acetophilum	2	+	+	+	–	–	–	77
C. limosum	17	+	+	+	–	–	–	61
C. multifermentans	7	+	+	+	–	–	–	73, 77
C. oedematicus	1	+	+	+	–	–	–	322
C. novyi	2	+	+	+	–	–	–	77
C. pasteurianum	3	+	+	+	–	–	–	77, 322
C. pseudofallax	1	+	+	+	–	–	–	77
C. putrificum	2	+	+	+	–	–	–	77
C. ramosus	17	+	+	+	–	–	–	143
C. rubrum	2	+	+	+	–	–	–	77
C. spheroides	1	+	+	+	–	–	–	322
C. sordelli	17	+	+	+	–	–	–	77, 274
C. sporogenes	2	+	+	+	–	–	–	77, 322
C. tetani	1	+	+	+	–	–	–	77
C. tetanomorphum	1	+	+	+	–	–	–	322
C. tyrobutyricum	1	+	+	+	–	–	–	77
C. fallax	2	–	+	+	+	+	–	77
C. pectinovorum	2	–	+	+	+	+	–	77, 322
C. perfringens	7	–	+	+	+	+	–	77, 322, 380
C. perfringens	1	–	1.0	1.6	1.0	0.65	–	221
C. perfringens	1	–	1.0	1.6	0.97	0.99	–	296
C. pseudofallax	1	–	+	+	+	+	–	77
C. innocuum	2	–	+	+	–	–	+	77
C. paraputrificum	2	–	+	+	–	–	+	77
C. septicum	1	–	+	+	–	–	+	77
C. tertium	2	–	+	+	–	–	+	77

following discussion we have tried to group these organisms according to the different types of peptidoglycans. The coryneform organisms show the greatest variation of peptidoglycan type of all bacterial families. Twenty-eight different types occur and almost half of them are found only among the coryneform bacteria. (Yamada and Komagata [429] have qualitatively studied the amino acid composition of a great number of cell walls of various coryneform bacteria. A part of their data disagrees with our findings compiled in Tables 31, 32, 33, and 34. They did not find Orn, Hsr, Hyg, Dab, Ser, and Thr. This may be due to nonideal conditions for chromatography.)

Variation A1γ. A great number of coryneform bacteria belong to the directly cross-linked, m-Dpm-containing peptidoglycan type. They are listed in Table 31. Using this peptidoglycan type as a basis and taking into consideration other features such as relation to oxygen and occurrence of vegetative life cycles, one can obtain further subdivisions (F. Fiedler, Ph.D. thesis, Technical University, Munich, 1971).

A uniform group is formed by the facultative anaerobic corynebacteria. They do not reveal a marked life cycle and their cell walls are distinguished by having m-Dpm and arabinogalactan. This group comprises primarily the human and animal pathogenic corynebacteria, but also some nonpathogenic strains. This group meets very well the definition of the genus *Corynebacterium sensu stricto* described

TABLE 31. *Coryneform organisms with a directly cross-linked, m-Dpm containing peptidoglycan*[a]

Group	No. of strains	Culture collection strain no.[b]	Arabinose and galactose[c]	Reference
I *Corynebacterium bovis*	1	ATCC 7715	+	74
C. callunae	1	ATCC 15991	0	101
C. cutis commune	1		0	23
C. diphtheriae	3		+	101, 69
C. equi	1	NCTC 1621	+	69
C. fascians	8	ATCC 12974, 12975	+	101, 69
C. herculis	1		0	429
C. hoagii	2	ATCC 13868	0	429
C. hoffmannii	1		+	23
C. hydrocarboclastus	4	ATCC 15108, 15592	+	Fiedler[d], 429
C. lilium	1	ATCC 15990	+	101
C. melassecola	1	ATCC 17966	+	Fiedler
C. nephridii	1	ATCC 11425	0	101
C. paurometabolum	1	ATCC 8368	+	101
C. petrophilum	1	ATCC 19080	0	Fiedler
C. pseudodiphtheriticum	1	ATCC 10700	+	101
C. pseudotuberculosis	1	ATCC 809	+	101
C. renale	1	NCTC 7448	+	69
C. rubrum	1	ATCC 14898	+	101
C. striatum	1	ATCC 6940	+	101
C. ulcerans	1	NCTC 7910	+	69
C. vesiculare	1	ATCC 11426	0	Fiedler
C. xerosis	5	ATCC 373, 7094, 7711, 9016	+	101, 74
M. flavum	2	ATCC 10340	+	335
II *Arthobacter albidus*	1	ATCC 15243	+	101
A. hydrocarboglutamicus		ATCC 15583	0	Fiedler
A. roseoparaffinus	1	ATCC 15584		Fiedler
Arthrobacter sp.	14	ATCC 19140, NCIB 9864	0	101
A. variabilis	2	ATCC 15753, NCIB 9455	+	101
A. viscosus	3	NCIB 10261, ATCC 15294, 15583	0	101
"Micrococcus" glutamicus	8	ATCC 13058, 13059, 13060, 13268, 13287, 13223, 13761, 15035	0	101
Brevibacterium ammoniagenes	4	ATCC 6872, 15137, 15750	+	101, 429
B. butanicum	1	ATCC 21196	+	Fiedler
B. chang-fua	1	ATCC 14017	+	101
B. divaricatum	2	ATCC 14020	+	101
B. flavum	2	ATCC 13826, 14067	+	101
B. glutamigenes	1	ATCC 13747	+	101
B. immariophilum	1	ATCC 14068	+	101
B. ketoglutamicum	1	ATCC 15587	+	101
B. lactofermentum	2	ATCC 13655, 13869	+	101
B. paraffinoliticum	1	ATCC 21195	+	Fiedler
B. roseum	1	ATCC 13825	+	101
B. saccharolyticum	1	ATCC 14066	+	101
Brevibacterium sp.	2	ATCC 14649, 19165	+	Fiedler
B. stationis	1	ATCC 14403	+	101
B. taipei	1	ATCC 13744	+	101
B. vitarumen	1	ATCC 10234	+	101
III *B. linens*	10	ATCC 8377, 9172, 9174, 9175	–	101
B. leucinophagum	1	ATCC 13809	–	101
IV *L. monocytogenes*	10		–	233, Fiedler

[a] A1γ; Fig. 6.
[b] See footnote *a* in Table 20.
[c] The occurrence of arabinose and galactose in the cell walls was established either by Cummins (75) or by F. Fiedler in our laboratory. 0, Not determined.
[d] F. Fiedler, Ph.D. thesis, Technical University, Munich, 1971.

TABLE 32. *Distribution of peptidoglycans of subgroup A3 among coryneform bacteria*

Variation	Peptidoglycan type	Species	Fig.	Reference
A3α	L-Lys-Gly	*Arthrobacter* sp. ATCC 19717; *Brevibacterium acetylicum* ATCC 954	23	101
	L-Lys-L-Ala	*A. crystallopoietes* ATCC 15481	19a	210, 211
	L-Lys-L-Ala$_2$	*A. pascens* ATCC 13346	19a	101
	L-Lys-L-Ala$_3$	*A. globiformis* ATCC 8010, NCIB 8602, 9759	13a	101
	L-Lys-L-Ala$_4$	*A. ramosus* NCIB 9066, *A. ramosus* NCIB 13727	13a	101
	L-Lys-L-Thr-L-Ala	*Arthrobacter* sp. NCIB 9423	25a	101
	L-Lys-L-Thr-L-Ala$_2$	*A. citreus* ATCC 11624	26a	101
	L-Lys-L-Thr-L-Ala$_3$	*Arthrobacter* sp.; *C. rathayi*; *Corynebacterium* sp. ATCC 21188	26a	101, Fiedler et al.[a]
	L-Lys-L-Ala-L-Thr-L-Ala	*A. aurescens* ATCC 13344; *A. histidinolovorans* ATCC 11442; *A. ureafaciens* ATCC 7562, *Arthrobacter* sp. ATCC 19141; *B. helvolum* ATCC 19239; *C. ilicis* ATCC 14264	26b	101; Fiedler et al.
	L-Lys-L-Ser-L-Thr-L-Ala	*A. polychromogenes* ATCC 15216; *A. globiformis* NCIB 8717; *A. oxydans* ATCC 14358, 14359; *Arthrobacter* sp. NCIB 9666; *B. album* ATCC 15111; *B. ammoniagenes* ATCC 6871; *B. cerinum* ATCC 15112; *B. helvolum* ATCC 11822; *C. alkanum* ATCC 21194	26c	101; Fiedler et al.
	L-Lys-L-Ser-L-Ala$_{2-3}$	*A. atrocyaneus* ATCC 13752	25b	156
A3γ	L,L-Dpm-Gly	*A. simplex* ATCC 6946, 13727, NCIB 9770; *Arthrobacter* sp. ATCC 14709	15a	101, 429
	L,L-Dpm-Gly$_3$	*A. tumescens* ATCC 9647	27a	101

[a] F. Fiedler, K. H. Schleifer, and O. Kandler, J. Bacteriol. 113:8–17.

by Barksdale (33). *Microbacterium flavum* belongs to this group, since not only the peptidoglycan type is identical but also arabinose and galactose are found in its cell walls. *M. thermosphactum* does not belong to this group, since its cell walls do not contain arabinose and galactose and the GC content (36%) is also atypical (63a). The relationship of the plant pathogenic species *C. fascians* to this group is uncertain. This organism contains m-Dpm, arabinose, and galactose in its cell walls, but there are several reasons to believe that this strain should be removed from the genus *Corynebacterium*. Conn and Dimmick (64), Lacey (212), and Ramamurthi (311) suggested that it be included in the genus *Nocardia*, whereas Cummins (69) recommended that it should be placed in the genus *Mycobacterium*. Either suggestion would agree with the cell wall composition found, since the strains of both genera contain m-Dpm, arabinose, and galactose in their walls (vide infra).

A second group of m-Dpm-containing coryneform bacteria differs from the first group by its strictly aerobic growth. Most of these organisms were hitherto included in the genus *Brevibacterium* and some of them in the genus *Arthrobacter* or *Micrococcus*. In contrast to the typical strains of *Arthrobacter*, they do not show a complete life cycle. Most of these strains also contain arabinose and galactose in their cell walls as do the strains of the first group (Table 31).

Interestingly enough, however, strains of *B. linens* do not contain these two sugars in their cell walls and may be separated into a third group. Further studies are therefore necessary to explore the taxonomic position of the second and third groups.

The genus *Listeria* is distinguished from the other m-Dpm-containing coryneform bacteria by morphological and physiological features.

TABLE 33. *Distribution of peptidoglycans of subgroup A4 among coryneform bacteria*

Variation	Peptidoglycan type	Species	Fig.	Reference
A4α	L-Lys-D-Asp	*Brevibacterium acetylicum* ATCC 953 *Kurthia zopfii* ATCC 6900, 10538	9	Cziharz;[a] Fiedler, *unpublished data*
	L-Lys-D-Glu	*B. incertum* ATCC 8363; *B. sulfureum* ATCC 19098	20a	Cziharz
	L-Lys-Ala-Glu	*Arthrobacter citreus* ATCC 21040, 21422; *A. nicotianae* ATCC 15236; *A. sp.* NCIB 9863; 2 *Arthrobacter* sp.;*B. fuscum* ATCC 15993; *B. liquifaciens* ATCC 14929	16b	101
	L-Lys-D-Ser-D-Asp	*B. liticum* ATCC 15921, *Corynebacterium manihot* NCIB 9097	24a	101
A4β	L-Orn-D-Asp	*Cellulomonas flavigena* ATCC 482	9	Fiedler and Kandler, *unpublished data*
	L-Orn-D-Glu	*Cellulomonas biazotea* ATCC 486; *C. cellasea* NCIB 8078; *C. fimi* ATCC 484; 15724; *C. gelida* ATCC 488;· *C. uda* ATCC 491; *C. subalbus* NCIB 8075	20a	Fiedler and Kandler, *unpublished data*
A4γ	m-Dpm-D-Asp_2	*A. duodecadis* ATCC 13347	29b	Bogdanovsky et al., *manuscript in preparation*
	m-Dpm-D-Glu_2	*Arthrobacter* sp. 1, NCIB 9859, 9860, 9861	17b	3g

[a] B. Cziharz, Diplom thesis, Technical University, Munich, 1969.

Moreover, the GC content of *Listeria* (38%) is quite different from that of human or animal pathogenic corynebacteria with 51 to 58% (141). From studies on the numerical taxonomy of *Listeria* (84) and from metabolic studies (251), it was concluded that *Listeria* and *M. thermosphactum* show a closer relationship to the family *Lactobacillaceae* than to the family *Corynebacteriaceae*. With regard to the GC content there could be a close relation between *Listeria* and streptococci (141). But against this can be set the fact that m-Dpm does not occur in streptococci (*see above*). Obviously the relationship of *Listeria* is not yet clear.

Variation A3α. Strains containing peptidoglycans of variation A3α form a rather uniform group, although they show a great number of different types of interpeptide bridges (Table 32, Fig. 25). Most of these strains belong to the genus *Arthrobacter* and are distinguished by strictly aerobic growth and a complete life cycle. This group conforms to the description of the genus *Arthrobacter* by Conn and Dimmick (64). Some strains hitherto classified as *Brevibacterium* or *Corynebacterium* (Fig. 26) are also included within this group since they contain the same variation of peptidoglycan.

Variation A3γ. Strains of *Arthrobacter simplex* and *A. tumescens* (Fig. 27) can be united into a further group of coryneform organisms. They also show a marked life cycle and are strictly aerobic, but their peptidoglycan types belong to variation A3γ (Table 32). L,L-Dpm occurs instead of L-Lys in position 3 of the peptide subunit. The interpeptide bridges consist of a single or several Gly residues (Fig. 15a, 27a). The proposal to classify *A. simplex* and *A. tumescens* within a distinct group is supported by previous evidence which separates both species from the main group of arthrobacters (84, 121). The occurrence of an identical peptidoglycan type and a similar GC content (70–74%) may indicate a possible relationship between these arthrobacters and certain aerobic actinomycetes (*see below*). L,L-Dpm and Gly were also found in cell walls of anaerobic corynebacteria. The amino acid composition of cell walls of *C. acnes* ATCC 6919, 6922, and 11828 and of *C. parvum* ATCC 11829 were qualitatively studied by Werner and Mann (410) and that of *C. anaerobium* by Nguyen-Dang (270). The qualitative amino acid compo-

TABLE 34. *Distribution of peptidoglycans of group B among coryneform bacteria*

Variation	Peptidoglycan type	Species	Fig.	Reference
B2β	[L-Hsr] D-Glu—D-Orn	*Brevibacterium albidum* ATCC 15831; *B. citreum* ATCC 15821; *B. luteum* ATCC 15830; *B. pusillum* ATCC 19096, 19097; *Corynebacterium betae* ATCC 13437; *C. flaccumfaciens* ATCC 6887, 12813, 23827; *C. poinsettiae* NCPP 177, ATCC 9682	11a	101, 286, 288
	[L-Hsr] D-Glu-Gly—D-Orn (Hyg)	*Arthrobacter terregens* ATCC 13345; *B. superdae* ATCC 19272; *C. barkeri* ATCC 15954; *Microbacterium liquifaciens*	11a	101, 335
B2γ	[L-Dab] D-Glu-D-Dab	*B. helvolum* ATCC 13715, 4 *B. helvolum* (Komagata); *C. aquaticum* ATCC 14665; *C. insidiosum* NCPP 1110, ATCC 10253; *C. mediolanum* ATCC 14004; *C. michiganense* ATCC 492, 4450, 7429, 7433, 10202, 4 strains from Dr. Stolp; *C. sepedonicum* NCPP 378 ATCC 9850; *C. tritici* NCPP 471	11c	101, 289, 291; Komagata, *personal communication*
B1α	[L-Lys] D-Glu-Gly—L-Lys (Hyg)	*M. lacticum* ATCC 8080, *C. laevaniformis* ATCC 15953	10a	101, 343
B1β	[L-Hsr] D-Glu-Gly_2—L-Lys (Hyg)	*B. imperiale* ATCC 8365		
B1γ	[L-Glu] D-Glu-Gly_2—L-Lys (Hyg)	*Arthrobacter* sp. J 39 (from Keddie)	10b	81
B1δ	[L-Ala] D-Glu-Gly-L-Lys	*Erysipelothrix rhusiopathiae*	10c	Fiedler and Kandler, *manuscript in preparation*

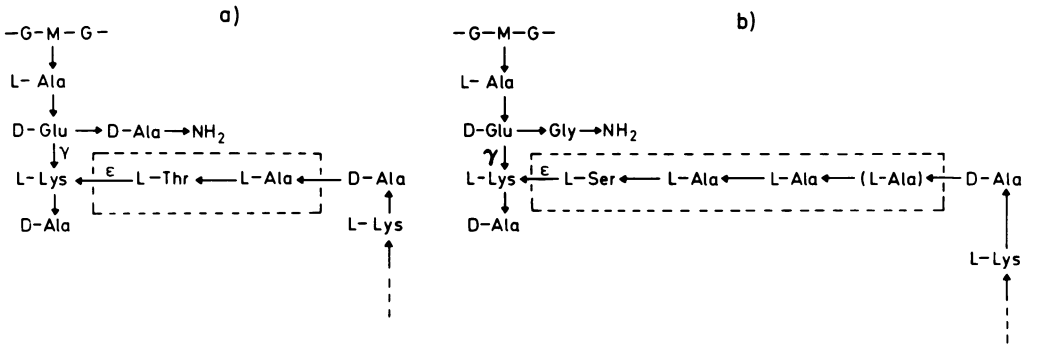

FIG. 25. *Fragments of the primary structures of peptidoglycans containing* D-*alanine amide and glycine amide as substituents of the* α*-carboxyl group of* D-*Glu (A3*α*). (a)* L-*Lys-*L-*Thr-*L-*Ala, Arthrobacter sp. NCIB 9423; (b)* L-*Lys-*L-*Ser-*L-Ala_{2-3}, *A. atrocyaneus ATCC 13752.*

sition was in all cases the same as that found in cell walls of many propionibacteria. Thus, the cell wall analyses seem to support the proposals of Douglas and Gunter (95), Gutierrez (131), and Moore and Cato (259) to transfer these anaerobic strains from the genus *Corynebacterium* to the genus *Propionibacterium*. More recent studies, however, do not favor such a

transfer (162a, 432). In particular the different GC content of anaerobic corynebacteria (47–58%) and propionibacteria (66–70%) and some physiological features speak against a close relationship of these two groups (408, 410, 411). There is also a rather low level of DNA homology (10–20%) between the anaerobic corynebacteria and classical propionibacteria (162a). It might be possible that the peptidoglycan of these organisms differs in a similar way from that of the propionibacteria as that of *Arachnia propionica* (vide infra). However, strains of *Arachnia propionica* show no DNA homology to either the anaerobic corynebacteria or the classical propionibacteria (162a).

Variation A4α. Variation A4α which contains L-Lys in position 3 of the peptide subunit was found in four different peptidoglycan types (Table 33). Most of the strains belong to the genera *Brevibacterium* and *Arthrobacter*. The exceptions are *C. manihot* and *Kurthia zopfii*. More physiological and genetic data are necessary to elucidate the relationships within this group.

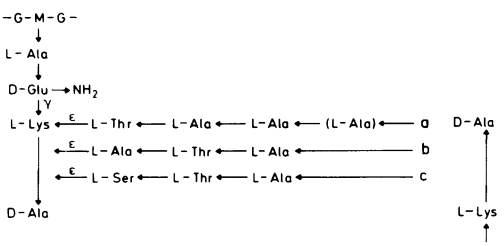

FIG. 26. *Fragments of the primary structures of threonine-containing peptidoglycans of coryneform organisms (A3α). (a) L-Lys-L-Thr-L-Ala$_{2-3}$; (b) L-Lys-L-Ala-L-Thr-L-Ala; (c) L-Lys-L-Ser-L-Thr-L-Ala.*

Variation A4β. This variation has only been found in the genus *Cellulomonas* (Table 33). This genus is characterized by the ability to decompose cellulose and to produce acid from carbohydrates. These features together with the distinct peptidoglycan types justify the genus *Cellulomonas* despite the objections of Jensen (161). With the exception of *C. flavigena* which contains the L-Orn-D-Asp type peptidoglycan, all the other species contain the L-Orn-D-Glu type (F. Fiedler, and O. Kandler, Arch. Microbiol., *in press*). (The amino acid composition of cell walls of *C. fimi*, *C. gelida* ATCC 488, *C. flavigena* ATCC 482, and *C. biazotea* ATCC 486 were qualitatively studied by Sukapure et al. (372). Their results were not in agreement with our findings. In the case of *C. fimi* and *C. gelida* ATCC 488, they found both Orn and Lys; in the case of *C. flavigena* ATCC 482 and *C. biazotea* ATCC 486, they did not find any diamino acid. We have obtained a subculture of the latter strain from M. Lechevalier, New Brunswick, and confirmed the presence both of Asp and of L-Orn in the cell walls. Physiological studies, however, have shown that this strain behaves as a typical *C. flavigena*. To clarify this discrepancy, a new subculture of *C. biazotea* ATCC 486 was obtained from the American Type Culture Collection. The determination of the peptidoglycan type corroborated our previous findings that *C. biazotea* ATCC 486 contains a L-Orn-D-Glu type.)

Variation A4γ. Peptidoglycans of variation A4γ were only detected in some coryneform bacteria and a few micrococci of doubtful taxonomic position (Table 33; 39). The occurrence of such a unique peptidoglycan type among a small group of strains makes a relationship between these strains quite likely. But further

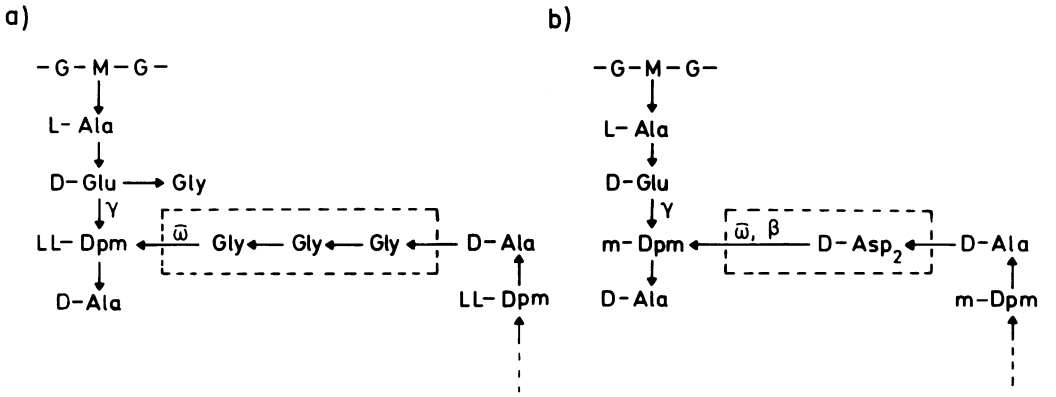

FIG. 27. *Fragments of the primary structures of Dpm-containing peptidoglycans (A3γ and A4γ) of coryneform organisms. (a) L, L-Dpm-Gly$_3$, A. tumescens ATCC 9647; (b) m-Dpm-D-Asp$_2$, A. duodecadis ATCC 13347.*

studies are necessary to classify these strains properly.

Group B. With one exception (*Butyribacterium rettgeri, see below*) peptidoglycan types of group B are met only among coryneform organisms. Seven different peptidoglycan types are found (Table 34). In particular the plant pathogenic corynebacteria show peptidoglycan types of group B. These findings support the proposals of several authors (33, 69, 82, 84, 134) to remove the plant pathogenic corynebacteria such as *C. betae, C. flaccumfaciens, C. insidiosum, C. michiganens, C. poinsettiae, C. sepedonicum*, and *C. tritici* from the genus *Corynebacterium*. Da Silva and Holt (82) suggested grouping these organisms with strains labeled *Microbacterium lacticum*. This would tally with the peptidoglycan structure, since the latter species and the closely related *M. liquifaciens* (318, 319) show a peptidoglycan structure quite similar to that of plant pathogenic corynebacteria (335, 343).

Erysipelothrix rhusiopathiae also contains a peptidoglycan of cross-linkage group B (Table 34), but with regard to other properties this species is quite different from typical plant pathogenic corynebacteria. It is animal and human pathogenic, grows under microaerophilic conditions, and contains no catalase. To clarify the relationship of *E. rhusiopathiae* to other bacteria, more genetic (e.g., GC content) and biochemical (e.g., fermentation pattern) data are necessary. But, on the basis of the peptidoglycan types, there is certainly no relationship between *E. rhusiopathiae* and animal pathogenic corynebacteria, *Kurthia* or *M. thermosphactum*, as suggested by Davis et al. (84).

Family Propionibacteriaceae. The family *Propionibacteriaceae* comprises anaerobic and aerotolerant species which are generally catalase-positive. They grow as irregular-shaped rods like coryneform organisms. From the three genera belonging to this family, the peptidoglycan composition of two genera, *Propionibacterium* and *Butyribacterium*, was studied.

Genus Propionibacterium. The amino acid composition of cell walls of various propionibacteria has been studied qualitatively (76, 380) and quantitatively (8). Studies on the primary structure of the peptidoglycan were carried out in our laboratory (345). Two different peptidoglycan types were found (Table 35). *P. shermanii* and *P. freudenreichii* contain the directly cross-linked peptidoglycan type with m-Dpm in position 3. In all the other species studied, the L,L-Dpm-Gly type (Fig. 17a) was observed. The distribution of these two types agrees with the different morphology (49) and the distinct physiological behavior of *P. shermanii* and *P. freudenreichii* (229).

TABLE 35. *Distribution of peptidoglycan types in the genus Propionibacterium*

Peptidoglycan type	Species	Reference
m-Dpm-direct[a]	*Propionibacterium shermanii* NCIB 5964 *P. shermanii* *P. freudenreichii* ATCC 6207	76, 345
L,L-Dpm-Gly[b]	*P. arabinosum* ATCC 4965, NCIB 5958 *P. jensenii* ATCC 4867, NCIB 8069 *P. pentosaceum* ATCC 4875, NCIB 8070 *P. petersonii* ATCC 4870, NCIB 5962 *P. rubrum* ATCC 4871, NCIB 8901 *P. thoenii* ATCC 4874 *P. zeae* ATCC 4964	8, 76, 345, 380

[a] Depicted in Fig. 6.
[b] Depicted in Fig. 15a.

Genus Butyribacterium. This monotypic genus was placed in the family *Propionibacteriaceae*. The species *B. rettgeri*, however, is quite distinct from typical propionibacteria. It is, in contrast to propionibacteria, catalase-negative and ferments carbohydrates, not to propionic acid, acetic acid, and carbon dioxide, but to butyric acid, acetic acid, and only small amounts of carbon dioxide. Moore and Cato (260) have considered *B. rettgeri* as a synonym of *Eubacterium limosum*.

Studies on the cell wall composition (249) and the amino acid sequence of the nucleotide-activated peptidoglycan precursor (250) have already shown that this species contains a peptidoglycan which is quite different from that of propionibacteria. Recent studies on the primary structure of the peptidoglycan have demonstrated that it belongs to group B (Fig. 11a; 129). This indicates a possible relationship to certain catalase-negative coryneform bacteria with a similar peptidoglycan type, e.g., *Erysipelothrix rhusiopathiae*.

Order Actinomycetales. Organisms belonging to the order *Actinomycetales* are distinguished morphologically from strains of other bacterial orders. The *Actinomycetales* often form a characteristic mycelium and multiply by means of special spores. Despite this similarity to the fungi, they are true bacteria (217). The order *Actinomycetales* can be divided into two

major groups based on their relation to oxygen: the aerobic and the anaerobic or facultative anaerobic actinomycetes.

Aerobic actinomycetes. The aerobic Actinomycetes are primarily classified on the basis of morphology and on the chemical composition of the cell walls (68, 69, 219, 320). Based on the cell wall composition, one can separate these aerobic actinomycetes into four groups. In Table 36 the various genera belonging to these four groups are listed. The taxonomic significance of the various generic names found in Table 36 has been critically reviewed by a number of authors (217, 218, 308, 423). With the exception of the genus *Mycobacterium* and various strains of the genus *Nocardia*, all the other genera produce a characteristic mycelium and special spores. These morphologically advanced actinomycetes were grouped together by Lechevalier and Lechevalier (218, 219) as *Euactinomycetes*.

Almost all of the studies on the chemical composition of the cell walls of these organisms are restricted to qualitative amino acid analyses (34, 46, 68, 69, 76, 218, 219, 225, 257, 372, 375, 428). The primary structure of the peptidoglycans has been determined in only a very few cases. Strains of the genus *Streptomyces* were studied in greater detail, and the amino acid sequence was established in two cases (19a, 221, 267). L,L-Dpm occurs in position 3 of the peptide subunit, and the cross-linkage is mediated by a glycine residue (Fig. 15a). From all the other genera mentioned in the first group of Table 36, the presence of L,L-Dpm and Gly in the cell walls was qualitatively determined. Based on these findings, it seems very likely that the peptidoglycan type is identical with that of *Streptomyces*. As mentioned earlier, the same peptidoglycan type was found in the *A. tumescens/A. simplex* group. This and other criteria (e.g., similar GC content and elongated

TABLE 36. *Distribution of suggested peptidoglycan types within aerobic actinomycetes and related organisms*

Suggested peptidoglycan type	Arabino-galactan	Genera	Reference
L, L-Dpm-Gly[a]	–	*Actinopycnidium*	219, 428
		Actinosporangium	219, 428
		Chainia	219, 428
		Elytrosporangium	219
		Intrasporangium	219, 372
		Microellobosporia	34, 219, 428
		Sporichthya	219
		Streptomyces	2, 19a, 46, 68, 76, 219, 221, 267, 375
m-Dpm-direct (m-HyDpm) plus Gly	–	*Actinoplanes*	34, 219, 373, 375, 428
		Amorphosporangium	34, 219, 373, 428
		Ampullariella	219, 285, 375, 428
		Dactylosporangium	219
		Micromonospora	34, 68, 76, 219, 398, 428
		Pilimelia	375
m-Dpm-direct[b]	–	*Actinobifida*	219
		Actinomadura	219
		Dermatophilus	34, 219
		Geodermatophilus	219, 225
		Microbispora	34, 219, 375, 428
		Micropolyspora	34, 219, 375, 428
		Planomonospora	219
		Thermoactinomyces	219
	+	*Mycobacterium*	2, 34, 68, 76, 78, 180, 181, 206, 255, 295, 372, 420
		Nocardia	34, 76, 219, 257
		Pseudonocardia	34, 138, 219

[a] See Fig. 15a.
[b] See Fig. 6.

and sometimes branched cells) may indicate a certain relationship between the L,L-Dpm-containing aerobic actinomycetes and the *A. tumescens/A. simplex* group.

A second group of aerobic actinomycetes contain Gly, as well as Ala, Glu, and m-Dpm in their cell walls. m-Dpm can be replaced partly or completely by 2,6-diamino-3-hydroxypimelic acid (285, 375, 428). It occurs predominantly as meso-diamino-threo-3-hydroxypimelic acid, but a small proportion of L,L-diamino-erythro-3-hydroxypimelic acid is also found (290). Up to now there is nothing known about the primary structure of this peptidoglycan. Gly may be bound to the α-carboxyl group of D-Glu, as in the case of *M. luteus*, or may be involved in the cross-linkage or may replace L-Ala bound to muramic acid. Preliminary results in our laboratory have shown that the latter is probably the case for the peptidoglycans of *Actinoplanes* and *Micromonospora*.

Other aerobic actinomycetes contain only Ala, Glu, and m-Dpm in their peptidoglycan. The genera showing this amino acid pattern are included in group 3 of Table 36. Although the chemical composition of the cell walls was only qualitatively determined, it seems likely that the peptidoglycan is cross-linked in a direct way (variation A1γ). Therefore, these organisms have probably the same peptidoglycan type as strains of the genus *Mycobacterium* but they do not contain arabinogalactan in their walls.

The chemical composition of cell walls of various mycobacteria (*M. phlei*, *M. rhodochrous*, *M. tuberculosis*, *M. bovis*, *M. smegmatis*, *M. lepraemurium*, *Mycobacterium* sp., and murine leprosy bacillus) has been studied qualitatively (2, 34, 76) and quantitatively (78, 180, 181, 255). The cell walls always contained the amino acids Ala, Glu, m-Dpm and the monosaccharides arabinose and galactose. In the case of *M. smegmatis* and *M. tuberculosis*, the primary structure of the peptidoglycan was established (206, 295, 420). The peptide subunits are directly cross-linked, and, as in the case of *C. diphtheriae*, both the α-carboxyl group of D-Glu and the carboxyl group of m-Dpm which is not involved in a peptide linkage are amidated. In the peptidoglycan of *M. tuberculosis* H37 Rv, however, a small percentage of the D-glutamic acid residues are not substituted by an amide but by glycine (206). The muramic acid residues in the peptidoglycans of all mycobacteria studied so far (*M. smegmatis*, *M. kansasii*, *M. tuberculosis*, and *M. phlei*) are not *N*-acetylated as is usually the case, but *N*-glycolylated (3, 25, 183). *N*-glycolylmuramic acid was also detected in the cell walls of *Nocardia kirovani* (130). In L,L-Dpm-containing peptidoglycans, such as that of *Streptomyces albus* and *Clostridium fermentans*, the usual *N*-acetylmuramic acid was found (25). Whether the occurrence of *N*-glycolylmuramic acid is restricted to mycobacteria, certain *Nocardia*, and *Micromonospora* (398) remains to be seen.

The amino acid composition of cell walls of about 60 strains of 28 species of *Nocardia* has been qualitatively determined (34, 69, 76, 219, 257). In most of the strains Ala, Glu, and m-Dpm were found, and the occurrence of the directly cross-linked, m-Dpm-containing peptidoglycan type, as in the case of mycobacteria and animal and human pathogenic corynebacteria, seems to be likely. In addition to having an identical peptidoglycan, they also contain arabinose and galactose in their cell walls. The same is true for the cell walls of *Pseudonocardia* (138). In some of the *Nocardia*, however, the m-Dpm is replaced by L,L-Dpm and no arabinose and galactose are present in the cell walls. The taxonomic position of these species (*N. alba*, *N. flava*, *N. gardneri*, *N. mesenterica*, *N. rubra*, *N. salmonicolor*, and several strains of *N. astroides*) is uncertain. The close relationship among *Corynebacterium*, *Mycobacterium*, and *Nocardia* is primarily based on the similar chemical compositions of their cell walls, in particular on the occurrence of the same peptidoglycan type in association with arabinogalactan (33). The arabinogalactan polymer is probably responsible for the serological cross-reaction of these three genera (67). Another characteristic property of these organisms is the occurrence of long-chain fatty acids which have been found in ester linkage with arabinose in both mycobacteria (25, 55, 182, 183, 295) and *Nocardia* (214). In *Corynebacterium*, the attachment of these fatty acids is not known yet (22, 407). The long-chain fatty acids are different in the three genera, e.g., *C. diphtheriae* contains corynemycolic and corynemycolenic acids (22, 353), *N. asteroides* and *N. rhodochorus* contain nocardic acids (157, 245), and *M. tuberculosis* contains mycolic acids (33). Thus, the different lipid composition may be useful for the separation of these three genera.

Family Actinomycetaceae. The family Actinomycetaceae comprises the anaerobic and facultative anaerobic actinomycetes. The information on the amino acid composition of the cell walls of these organisms is conflicting. In most of the studies only the qualitative amino acid composition has been determined (45, 56, 69, 71, 72, 76, 108, 119, 297, 298, 299). The amino acid composition has been quantita-

tively determined only in one study (91). Unfortunately, the cell walls used for this study were obviously contaminated with protein, and the results must be taken with caution.

Recently, we have studied in our laboratory the amino acid composition of cell walls of six species which were kindly supplied by G. H. Bowden, London. As shown in Table 37 there are three different types of peptidoglycan. Cell walls of *A. bovis* contain L-Lys as diamino acid and D-Asp in addition to Ala and Glu. The peptide patterns of two-dimensional chromatograms of partial acid hydrolysates showed that this peptidoglycan is of the L-Lys-D-Asp type as in many lactobacilli and some bifidobacteria. The same is true for *A. eriksonii*. This latter organism reveals the same type of fermentation as bifidobacteria (297) and should be transferred to the genus *Bifidobacterium* (258).

A. naeslundii, *A. israelii*, *A. odontolyticus*, and *A. viscosus* show a unique amino acid composition, Mur:$GlcNH_2$:Ala:Glu:Lys:Orn = 1:1:2:2:1:1. Such molar ratios have never been found in other organisms up to now. Preliminary results on the structure of this peptidoglycan indicate that it belongs to group B. The interpeptide bridge between the α-carboxyl group of Glu and the C terminus of D-Ala of an adjacent peptide subunit consists most likely of glutamyl-ornithine, whereas L-Lys occupies position 3 of the peptide subunit and does not take part in the cross-linkage.

Since peptidoglycan types of group B are only found within coryneform bacteria (*Microbacterium*, *Corynebacterium*, *Brevibacterium*) but never in bifidobacteria, a relationship of the anaerobic actinomycetes (with exception of *A. bovis*) to the coryneform organisms seems to be more likely than to the bifidobacteria as suggested by Pine (297) and Prevot (309).

One of the main arguments of Pine for a closer relationship of these organisms to *Bifidobacterium* was the occurrence of Lys and Orn in the peptidoglycan of both groups of organisms. As indicated by our studies, however, the structures of the two types of peptidoglycan are quite different. In *B. bifidum* only 1 mole of diamino acid per mole of Glu is present and Lys and Orn replace each other in position 3 of the peptide subunit, whereas 1 mole each of Lys and Orn is present in the actinomyces strains and both of them occur at quite different positions. Here we have a very good example of how risky it is to draw conclusions prematurely from qualitative studies of the cell wall composition before knowing the primary structure.

Actinomycetales of uncertain taxonomic position. *Genus Arachnia.* Cell walls of *Arachnia propionica* contain L,L-Dpm and Gly like those of many propionibacteria (297). Since the fermentation pattern is also quite similar to that of propionibacteria, it was suggested that *A. propionica* be placed within the family *Propionibacteriaceae* (297). The quantitative studies of the amino acid composition of the cell walls of *A. propionica* (Table 37), however, showed a remarkable difference from that of the peptidoglycan of propionibacteria. Whereas the latter contains 1 mole of Gly and 2 moles of Ala per mole of Glu, in *A. propionica* 2 moles of Gly

TABLE 37. *Amino acid composition of cell walls of anaerobic actinomycetes and Arachnia propionica*

Organisms	Muramic acid	Gluc-NH_2	Lysine	Ornithine	L,L-Dpm	Glutamic acid	Alanine	Aspartic acid	Glycine	Ammonia
Actinomyces bovis ATCC 13683	0.7	0.8	0.9			1.0	1.6	0.85		1.1
A. naeslundii ATCC 12104	0.8	0.7	0.7	1.0		1.7	1.7			1.1
A. odontolyticus NCTC 9935	1.0	1.5	1.1	1.0		2.1	1.9			1.2
A. odontolyticus WVU 482	1.0	1.3	1.0	1.0		2.05	1.9			1.2
A. israeli serotype 1, NCTC 4860	0.9	1.3	0.8	1.0		1.9	1.7			0.9
A. israeli serotype 2, C 65	1.0	2.1	1.0	1.0		2.1	2.1			1.3
A. viscosus serotype 1, ATCC 15987	0.95	1.3	1.1	1.0		2.1	1.9			1.3
A. viscosus serotype 2, WVU 371	0.9	0.8	0.85	1.0		1.7	1.6			1.1
Arachnia propionica ATCC 14157	0.75	0.6			1.05	1.0	1.65		2.05	1.3
A. eriksonii ATCC 15423	0.7	0.95	0.7	0.3		1.0	1.6	0.9		0.9

and 1 mole of Ala were found. Preliminary results on the primary structure of this peptidoglycan indicate that L-Ala in position 1 of the peptide subunit is replaced by Gly as in peptidoglycans of group B, but the cross-linkage is like that of group A. A single Gly residue connects two peptide subunits between L,L-Dpm and D-Ala, as in most species of the genus *Propionibacterium* (Fig. 15a).

The transfer of *A. propionica* to the family *Propionibacteriaceae* (297) seems to be justified also in respect to the peptidoglycan structure, since it contains peptidoglycan similar to that of many propionibacteria. At the same time the genus *Arachnia* may still be maintained since, in contrast to typical propionibacteria, catalase is absent and the peptidoglycan type shows a distinct deviation from the type found in the genus *Propionibacterium*.

Genus Rothia. Rothia is a monotypic genus with the single species *R. dentocariosus*. It was first isolated from carious dentine in humans by Onisi (276) and described as *Actinomyces dentocariosus*. In later studies it was described as *Nocardia dentocariosus* (325) and *N. salivae* (83). Since neither *Nocardia* nor *Actinomyces* appeared to be an appropriate generic name for this species, Georg and Brown (107) proposed the new genus *Rothia*, in the family *Actinomycetaceae*. Under anaerobic conditions, it is able to ferment glucose to lactic acid as a major product, but it does not grow anaerobically and is, in contrast to strains of *Actinomyces*, catalase-positive.

The cell wall composition of this organism was studied qualitatively, but the results do not agree completely. Ala, Glu, and Lys have always been found (297), but in one study Orn (107) and in another Asp (372) were also present. We obtained two strains of *R. dentocariosus* (D 10B and XPGA) from M. P. Lechevalier (Rutgers University, New Brunswick, N.J.) and studied the amino acid sequence of their peptidoglycans. Preliminary results show that both of them belong to the L-Lys-L-Ala_3 type (Schleifer, *unpublished results*). No Asp was found. Despite the fact that this type is rather common, it has not hitherto been found among *Actinomycetales*. This may justify the separate classification of *Rothia* but does yet not clarify its relationship to other gram-positive bacteria.

Genus Oerskovia. The motile nocardioid bacteria first described by Ørskov (278) are clearly not members of the genus *Nocardia*. Prauser et al. (308), therefore, proposed to place them in a separate genus *Oerskovia*. The only species known up to now is *O. turbata*. Qualitative cell wall analyses have shown that strains of *O. turbata* contain Asp in addition to Ala, Glu, and Lys (372). We obtained two strains of *O. turbata* (891 and Y 13-4) from M. P. Lechevalier (Rutgers University, New Brunswick, N.J.) and studied the amino acid sequence of their peptidoglycans. Like Sukapare et al. (372), we have found Ala, Glu, Lys, and Asp as amino acid constituents of their cell walls, but in addition one strain (Y 13-4) contains Ser and the other strain (891) Thr. Preliminary results indicate that the peptide subunits have the usual primary structure (L-Ala-γ-D-Glu-L-Lys-D-Ala), and the interpeptide bridges consist of the dipeptide β-Asp-Ser and β-Asp-Thr, respectively (Schleifer, *unpublished results*). Thus, strain Y 13-4 contains the peptidoglycan type L-Lys-Ser-Asp as found in *B. liticum* and *C. manihot*. Strain 891 contains a new peptidoglycan type L-Lys-Thr-Asp. These two types are quite similar. Both contain a β-linked Asp and a hydroxyamino acid in their interpeptide bridges. Further studies are necessary to prove if this is the case for all *Oerskovia* strains. If so the occurrence of Thr or Ser in these cell walls may be a good character for a further classification of the genus *Oerskovia*.

Order Caryophanales. The order *Caryophanales* comprises bacteria forming large trichomes. The chemical composition of the cell walls has been examined in one strain of *Caryophanon latum* and one of *Lineola longa*.

Genus Caryophanon. Caryophanon latum is a typical inhabitant of fresh cow dung. The cell walls of this peritrichously flagellated organism were isolated, and their chemical composition was qualitatively analyzed (35). Besides muramic acid and glucosamine, the common cell wall amino acids Ala, Glu, and Lys were found. It is not known if the peptidoglycan is cross-linked in a direct way or if an interpeptide bridge is present.

Genus Lineola. Lineola longa is now reclassified as *Bacillus macroides*. Preliminary studies on cell walls of two strains of *B. macroides* have yielded Lys as the predominant diamino acid and no m-Dpm as usually found in the peptidoglycan of bacilli (222). Examination of the same cell walls in our laboratory have shown that both strains of *B. macroides* contain the Lys-D-Asp type of peptidoglycan (*unpublished results*). This peptidoglycan type was found among bacilli only in *B. sphaericus* (*see above*).

Order Spirochaetales. The morphology of spirochaetes is quite different from that of other bacterial cells. The flexible, thin cell can be tightly coiled, and a very thin helical element, the so-called axial filament, is closely wrapped

around the cell. With regard to the Gram reaction, the spirochaetes are presumably gram-negative. Thus, it was expected that the peptidoglycan would show the directly cross-linked m-Dpm type. But already qualitative studies on cell walls of *Treponema reiteri* have yielded ornithine instead of m-Dpm as the major diamino acid (382). Recent studies on the ultrastructure and chemical composition of the cell wall of *Spirochaeta stenostrepta* have confirmed the presence of L-Orn as constituent of the peptidoglycan (166). Two layers of the cell wall of *Spirochaeta stenostrepta* were isolated and analyzed. The outermost of these two layers consists mainly of lipoprotein. The second layer was characterized as peptidoglycan. It is a thin structure or monolayer as in typical gram-negative organisms which retains the cylindrical and coiled shape of the cell. Studies on the amino acid sequence of the purified peptidoglycan revealed that the peptide subunits consist of tripeptides (L-Ala-γ-D-Glu-L-Orn) and tetrapeptides (L-Ala-γ-D-Glu-L-Orn-D-Ala). The peptide subunits are directly cross-linked (Fig. 28). The primary structure of the peptidoglycan resembles that of other gram-negative bacteria with the only difference being that m-Dpm is replaced by L-Orn.

Order Myxobacteriales. Since the myxobacteria are flexible cells, it has been assumed that the cell walls are quite different from those of eubacteria and that the peptidoglycan may be lacking. But even the first studies on the cell walls of myxobacterial strains showed that a peptidoglycan layer is probably present in their walls (7, 239). More detailed studies on myxobacterial cell walls were reported by Verma and Martin (397) and by White et al. (415). The

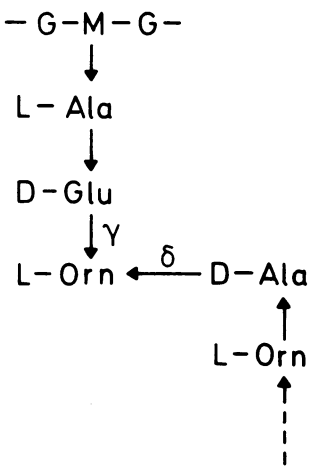

FIG. 28. *Fragment of the primary structure of the peptidoglycan of Spirochaeta stenostrepta.*

results demonstrated that in all species so far examined (*Cytophaga hutchinsonii*, *Sporocytophaga myxococcoides*, and *Myxococcus xanthus*), the directly cross-linked, m-Dpm-containing peptidoglycan type occurs (Fig. 6). In the case of *C. hutchinsonii* and *S. myxococcoides*, a discrete peptidoglycan layer was isolated (397). The vegetative cell walls of *M. xanthus*, on the other hand, completely disaggregated after treatment with trypsin and detergent. White et al. (415) suggested, therefore, that the rigid layer of the vegetative cell wall of *M. xanthus* is not a continous peptidoglycan layer but consists of patches of peptidoglycan separated by nonpeptidoglycan material. This patchlike arrangement may be related to the flexibility of the myxobacterial cell. In the case of *C. hutchinsonii* and *S. myxococcoides*, however, the flexibility is explained by the occurrence of "naked tubes of murein (peptidoglycan) monolayers" (397).

With the exception of the genus *Cytophaga*, all myxobacteria can form resting cells. These resting cells, the so-called microcysts, are produced from single vegetative cells. The microcysts are shorter than the vegetative cells and their cell walls are much thicker and inflexible. In both *Sporocytophaga* and *Myxococcus*, not only the vegetative cell walls but also the cell walls of microcysts were studied. Verma and Martin (397) explained the transition of the flexible, vegetative cell wall of *Sporocytophaga* to the rigid, thick microcyst cell wall by the superposition of several peptidoglycan layers. White et al. (415), on the other hand, found in *Myxococcus*, during the change from the vegetative cell to the microcyst, a temporary decrease in the cross-linkage of the peptidoglycan. They assume a high rate of turnover of the peptidoglycan during the formation of the microcyst and simultaneously some uncross-linked peptidoglycan appears. A newly synthesized extracellular layer of nonpeptidoglycan material serves to strengthen the microcyst wall. Moreover, the authors stated that "the patch-like arrangement of the peptidoglycan may be related to the change in shape when *M. xanthus* converts from the vegetative rod to the spherical cyst." They think that the nonpeptidoglycan areas perform a necessary role in the morphogenesis of the wall.

FINAL REMARKS

Taxonomic Implications of Other Cell Wall Polymers

Lipopolysaccharides. A good correlation between the structure of the lipopolysaccha-

rides and other taxonomic characteristics has been found among different groups of gram-negative bacteria (4–6, 127, 192, 399), especially within the *Enterobacteriaceae* (228, 272). Within the genera *Salmonella* and *Escherichia* (120, 228, 348) it was found that the lipopolysaccharides consist of a lipid portion, the so-called lipid A, and the polysaccharide portion. The lipid A portion of several *Salmonella* serotypes and of an *E. coli* strain appeared to be very similar or even identical (120). The polysaccharide portion, however, reveals a great variability. It consists of a central core, the so-called R core containing five basic sugars, and a variable portion, the O side chain which contains various sugars and carries all the antigenic specificity. The R core is named after the R mutants which are defective in the biosynthesis of the O side chains and synthesize only the central core of the lipopolysaccharide (226). The structure of the R core is the same or at least very similar in all *Salmonella* strains and it is different from, yet related to, that of *Shigella*. The structure of the R core, therefore, seems to be rather similar within each genus (347). Only within the heterogeneous group of *E. coli* are R cores with different structures found. Therefore, the enormous diversity in the sugar composition and arrangement of the lipopolysaccharide mainly reflect the variability of the peripheral O side chains. The latter are responsible for the distinct antigenic properties. Thus long before the structure of the lipopolysaccharide had been established, immunological variability was used for the classification of the *Enterobacteriaceae* (191). By this method more than 700 different "species" (serospecies) can be distinguished within the genus *Salmonella* alone. In certain cases, however, the conclusions drawn from the serological typing reactions can be misleading. Serological tests and chemical studies have shown that strains of different genera have identical or at least very similar O antigens (272, 412). Thus certain strains of *Salmonella*, *Arizona*, and *E. coli* show complete serological cross-reaction of their heat-stable somatic O antigens. (For more detailed information on this problem, see the excellent reviews by Lüderitz et al. [227, 228].)

Polysaccharides. There has been less-intensive work on the structure and serology of the cell wall polysaccharides of gram-positives than on the lipopolysaccharides of gram-negatives. The best studied cell wall polysaccharides among gram-positive bacteria are those of the streptococci. These cell wall polysaccharides form the basis for the serological grouping of the streptococci with the exception of groups D and N (213). The chemical composition and structure of these group-specific polysaccharides have been studied in various laboratories (79, 80, 186, 207, 246, 247, 266, 282, 360). There is a good correlation between the serological specificity of the group antigens and the chemical structure of these polysaccharides. Elliot et al. (97) have very recently suggested that the cell wall polysaccharides of hemolytic streptococci reveal a structure resembling that found in the lipopolysaccharides of *Enterobacteriaceae*. The rhamnose polymer is suggested as the counterpart of the R core of the lipopolysaccharide. But more chemical studies are necessary to demonstrate that all streptococcal cell wall polysaccharides contain the same or at least similar rhamnose polymers as core structure.

A few studies have been performed on the chemical structure of the cell wall polysaccharides of lactobacilli (132, 196) and mycobacteria (256).

Teichoic acids. Teichoic acids are found instead of or in addition to polysaccharides in the cell walls of many gram-positives. Teichoic acids are water-soluble polymers containing sugar, D-alanine residues, and either glycerol or ribitol phosphates. Most of the work on the structure and distribution of these polyol phosphate polymers has been carried out by Baddiley and coworkers. (See recent reviews or monographic treatments for further information regarding structural work [11–16, 19, 27].) The teichoic acids are also immunologically active polymers, and the occurrences of structurally and therefore serologically different teichoic acids are used in the classification of staphylococci and lactobacilli (197, 275, 335a, 355).

Teichuronic acids. In a few organisms such as *Micrococcus luteus* (284), *Bacillus subtilis* (160), *B. licheniformis* (149), and *B. cereus* (148a), another acidic polysaccharide is found in the cell walls, namely teichuronic acid. Teichuronic acid consists of glycosidically linked sugar and uronic acid residues (136, 149). The occurrence of this polymer may provide a good criterion for identifying the organisms in question. The distribution of this cell wall polymer, however, may not be widespread enough to be of greater taxonomic importance.

Lipids. The occurrence of significant amounts of lipid is typical of the cell walls of gram-negative bacteria and of some gram-positives, such as *Mycobacterium*, *Corynebacterium*, and *Actinomycetales*. The lipid composition, however, varies only little among gram-negatives (187). Moreover, environmental factors, such as growth temperature, composition of the growth medium, or age of the bacterial culture, influence the qualitative and quantitative composition of the lipids (152, 235), thus

limiting their taxonomic value. The patterns of long-chain fatty acids, however, in the cell walls of *Corynebacterium*, *Mycobacterium*, and *Nocardia* are rather constant within each genus but differ characteristically among the different genera. Thus, they may prove to be a valuable taxonomic characteristic (22, 33).

Taxonomic Relevance and Evolutionary Trends of Peptidoglycan Structure

Before considering the value of the peptidoglycan structure in taxonomy, we must know the prerequisites of a useful taxonomic marker. (i) It should show a widespread distribution in the group to be classified. (ii) It should be determined by as many genes as possible to attain a certain stability and to make a convergent formation of identical variations rather unlikely. (iii). It should show the possibility of genetic variability in small steps without appreciably changing the competitiveness or vitality of the organisms to conserve a great number of distinct variations in various directions. (iv) The variations should reveal derivative sequences, i.e., be of such kind that it is possible to recognize if a particular structure is primitive or highly developed.

Moreover, a taxonomic marker should not depend on growth stages or environmental factors and should be readily determinable to be routinely employed. However these last two properties are rather a matter of convenience than an absolute necessity for a good taxonomic characteristic. If the properties of a characteristic are dependent on growth phase or environmental factors, it is possible to take these limitations into consideration.

It is, for example, possible to harvest the organisms at a certain stage of growth or to avoid unfavorable environmental conditions. Laborious determination of characteristics may be circumvented if the presence of more easily determinable correlative traits has been established. Thereafter, analysis for these traits may suffice for purpose of classification. With regard to the peptidoglycan, the phenotypic alterations due to growth phase or environmental factors were already discussed in a previous chapter, and it was found that the peptidoglycan types are not seriously changed by these factors. The determination of the peptidoglycan structure is still too laborious for routine application, but a simplification of the procedure has been proposed in this article (*see above*) and a serological determination may be conceivable. We may now examine to what extent the chemical structure of the peptidoglycan fulfills the conditions of a good taxonomic characteristic.

(i) The peptidoglycan is a typical constituent of cell walls of almost all procaryotic cells and thus fulfills the first condition by its ubiquitous distribution. The basic structure of the glycan moiety is found in all wall-containing organisms. The more advanced organisms, however, show a simplified structure. Thus chitin, a main constituent of most fungal cell walls, lacks the lactic acid ether as well as the peptide moiety, and the cellulose of plant cell walls additionally lacks the amination. There is a clear deductive line recognizable at the highest level of taxonomic hierarchy: the progressive simplification (= reduction) from the peptidoglycan structure of the procaryotes to the chitin structure of fungi and the cellulose structure of plants clearly separates the kingdom of procaryotes and these two eucaryotic kingdoms. The use of the same principle for the construction of the main component of the cell walls throughout the "plant kingdom in sensu lato" indicates a monophyletic origin of all these organisms. We are well aware that some arguments favor for a polyphyletic origin of the bacteria, but it is very difficult to understand why diverse groups, which were never in genetic exchange with each other, should have invented the same principle for cell wall construction.

(ii) The second condition calls for a characteristic which is a result of multiple gene action. The formation of the peptidoglycan structure certainly depends on the action of many genes. At least 20 different enzymes are involved in the formation of a simple peptidoglycan structure, and most of the diverse peptidoglycan types differ from each other in more than one gene. This explains the rather stable character of a given peptidoglycan structure. No single-step mutations are known so far which lead to an altered peptidoglycan type. This genetic stability, furthermore, excludes the involvement of extrachromosomal genes in the fixation of the peptidoglycan type. The polygenic character of the different variations makes it rather unlikely that they have arisen by convergence. At most, the variations of the interpeptide bridges may be subjected to convergence more frequently.

(iii) In contrast to the stability of a peptidoglycan type for "short" periods (times during which one is able to investigate an organism), there has had to be a considerable mutational alteration of the chemical structure of the peptidoglycan of gram-positive bacteria during the course of evolution. This diversification of the chemical structure of the peptidoglycan was obviously not connected with a remarkable advantage or disadvantage of selection, since so

many different peptidoglycan types are still conserved. This fulfills, for the gram-positives, the third condition of a good taxonomic characteristic.

This does not apply to the gram-negatives, and they do not show the same chemical and structural variability of the peptidoglycan as the gram-positives. The peptidoglycan of the gram-negatives consists only of a monolayer, in contrast to the multilayered peptidoglycan of gram-positives. In such a simple monolayer, the variability of the cross-linkage may be restricted. Even the insertion of interpeptide bridges might distort its structure and therefore decrease the vitality and competitiveness of the organisms. Thus, a change in the peptidoglycan type of the "sophisticated" monolayer of a gram-negative bacterium would be most likely a disadvantage. This may be the reason why such alterations have not been conserved. Consequently, the peptidoglycan type is not a taxonomic characteristic for classification among the gram-negative bacteria.

(iv) An unequivocal phylogenetic relationship of the various peptidoglycan structures is not readily discernible, but rough deductive sequences are recognizable. A possible phylogenetic connection of the different cross-linkage variations is sketched in Fig. 29. It is based on the assumption that phylogenetic advancement is usually connected with a simplification (= reduction) and a loss of variability (= fixation). Thus the most advanced type of peptidoglycan should be characterized by the greatest possible simplification (from a chemical point of view), whereas the more primitive ones should contain additional trappings in abundant variations. This trend to an extensive chemical simplification of the basic structure is also discernible at a higher stage of evolution and was mentioned before in the comparison of the peptidoglycan of procaryotes and the main cell wall structures of fungi and plants.

The occurrence of multilayered peptidoglycans containing interpeptide bridges may represent a primitive stage. Therefore, we have placed subgroups A3 and A4 at the basis of our scheme (Fig. 29). These two subgroups contain the large majority of the peptidoglycan types and are represented by the majority of gram-positive, non-spore-forming bacteria including most of the fermenting organisms. Both subgroups reveal such an enormous ramification of peptidoglycan types that phylogenetic relationships among these different variations are not readily discernible. Thus, the condition expressed in this point, that the structure should reveal its primitive or deduced character, is not fulfilled here by the peptidoglycan. The great variability within the two subgroups A3 and A4 can be taken as a further indication of their primitive stage, since this variability discloses that the evolution of the peptidoglycan structure is at this point far from reaching a standstill.

A major side branch of these two subgroups has probably led to the formation of group B. Group B may have arisen only once, since it is restricted to a rather unique group of bacteria, viz, a relatively small number of coryneform

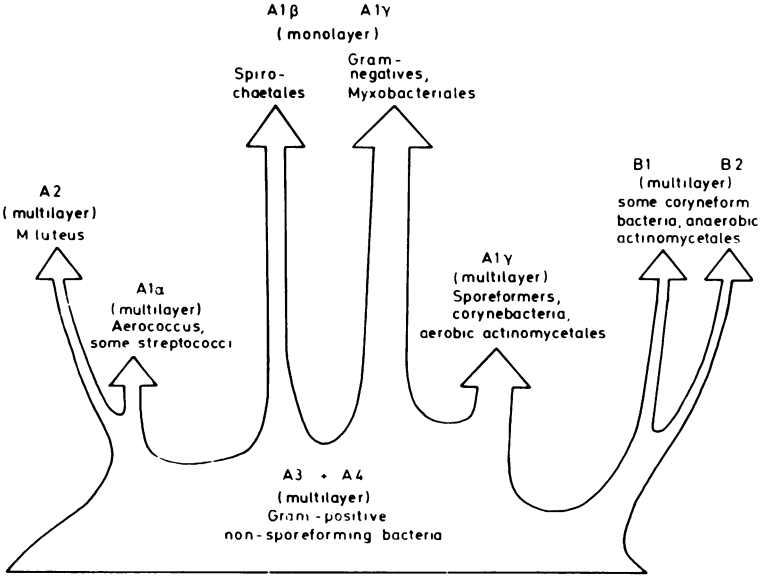

FIG. 29. *A possible phylogenetic relationship of peptidoglycan subgroups and variations.*

organisms and the anaerobic actinomycetes. Peptidoglycan types of group B which contain L-Lys or L-Orn in position 3 of the peptide subunit clearly reveal a close relationship with that of group A and signify a transition between these two groups of cross-linkages. Only after the cross-linkage between the carboxyl groups of D-Glu and D-Ala had evolved could the diamino acid at position 3 of the peptide subunit be replaced by monoamino acids without lethal consequences. Although we assume that the phylogenetic development proceeded from A to B, the reverse may have equally occurred.

Group B also shows a considerable diversity of variations and types, but the numbers of types are smaller than that within subgroups A3 and A4. Further evolution of group B might conceivably lead even to the omission of the amino acid in position 3 of the peptide subunit.

Whereas group B does not represent a simplification but reveals about the same complexity as subgroups A3 and A4, a simplification of the peptidoglycan composition and structure is found within peptidoglycans of group A. Both the directly cross-linked peptidoglycans of subgroup A1 and the polymerized peptide subunits of subgroup A2 are certainly chemically simpler than the peptidoglycans of subgroups A3 and A4, since the interpeptide bridges are omitted. Subgroup A2 is a special more advanced case of a directly cross-linked peptidoglycan. As mentioned earlier (*see above*) one or perhaps two additional enzymes are necessary to form these polymerized peptide subunits instead of a directly cross-linked peptidoglycan.

The most successful branch is the directly cross-linked m-Dpm-containing peptidoglycan (variation A1γ). Not only is it the most common peptidoglycan type, but also the most highly evolved procaryotes, such as myxobacteria and blue-green algae, reveal this type of peptidoglycan. The directly cross-linked, m-Dpm-containing type of peptidoglycan is found in two forms, each with a different ultrastructure. Hereby, the gram-positives have conserved the multilayer, whereas the gram-negatives have evolved the monolayer as the *Spirochaetales* have within the variation A1β. The arrangement of the peptidoglycans in a monolayer may represent the highest level of evolution. The simplification is so far advanced that even the supporting function of the peptidoglycan layer is hardly maintained. Additional components, such as lipoproteins (48) or proteins (415), are closely associated with the monolayered peptidoglycan to reinforce it.

Although we have derived a rough phylogenetic scheme from peptidoglycan structures, we are fully aware that such a monothetic system does not contain sufficient information to represent the finer details of phylogenetic development. Nevertheless, the knowledge of the various peptidoglycan structures and the possible phylogenetic implications will unquestionably contribute not only to the classification of gram-positive bacteria but also to the clarification of the phylogenetic relationships among the procaryotes.

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LITERATURE CITED

1. Abo-Elnaga, I. G., and O. Kandler. 1965. Zur Taxonomie der Gattung *Lactobacillus* Beijerinck. I. Das Subgenus *Streptobacterium* Orla Jensen. Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. 2. **119**:1-36.
2. Acharya, P. V. N., and D. S. Goldman, 1970. Chemical composition of the cell wall of H37a strain of *Mycobacterium tuberculosis*. J. Bacteriol. **102**:733-739.
3. Adam, A., J. F. Petit, J. Wietzerbin-Falszpan, P. Sinay, D. W. Thomas, and E. Lederer. 1969. L'acide N-glycolylmuramique, constituent des parois de *Mycobacterium smegmatis*: identification par spectrometrie de masse. Fed. Eur. Biochem. Soc. Lett. **4**:87-92.
4. Adams, G. A. 1971. Structural investigations on a cell-wall lipopolysaccharide from *Neisseria sicca*. Can. J. Biochem. **49**:243-250.
5. Adams, G. A., T. G. Tornabene, and M. Yaguchi. 1969. Cell-wall lipopolysaccharides from *Neisseria catarrhalis* Can. J. Microbiol. **15**:365-374.
6. Adams, G. A., C. Quadling, M. Yaguchi, and T. Tornabene. 1970. The chemical composition of cell-wall lipopolysaccharides from *Moraxella duplex* and *Micrococcus calco-aceticus*. Can. J. Microbiol. **16**:1-8.
7. Adye, J. C., and D. M. Powelson. 1961. Microcyst of *Myxococcus xanthus:* chemical composition of the wall. J. Bacteriol. **81**:780-785.
8. Allsop, J., and F. Work. 1963. Cell walls of *Propionibacterium species:* fractionation and composition. Biochem. J. **87**:512-519.
9. Araki, Y., T. Nakatani, R. Makino, H. Hayashi, and E. Ito. 1971. Isolation of glucosaminyl β (1-4)-muramic acid and phosphoric acid ester of this disaccharide from acid hydrolysates of peptidoglycan of *Bacillus cereus* AHU 1356 cell walls. Biochem. Biophys. Res. Commun. **42**:684-690.
10. Araki, Y., T. Nakatani, H. Hayashi, and E. Ito.

1971. Occurrence of non-N-substituted glucosamine residues in lysozyme-resistant peptidoglycan from *Bacillus cereus* cell walls. Biochem. Biophys. Res. Commun. **42**:691-697.
11. Archibald, A. R., and S. Heptinstall. 1971. The teichoic acids from *Micrococcus* sp. 24. Biochem. J. **125**:361-363.
12. Archibald, A. R., J. Baddiley, and J. G. Buchanan. 1961. The ribitol teichoic acid from *Lactobacillus arabinosus* walls: isolation and structure of ribitol glucosides. Biochem. J. **81**:124-134.
13. Archibald, A. R., J. Baddiley, D. Button, S. Heptinstall, and G. H. Stafford, 1968. Occurrence of polymers containing N-acetylglucosamine 1-phosphate in bacterial cell walls. Nature (London) **219**:855-856.
14. Archibald, A. R., J. Baddiley, and D. Button. 1968. The glycerol teichoic acid of walls of *Staphylococcus lactis* I3. Biochem. J. **110**:543-557.
15. Archibald, A. R., J. Baddiley, and G. A. Shankat. 1968. The glycerol teichoic acid from walls of *Staphylococcus epidermidis* I2. Biochem. J. **110**:583-588.
16. Archibald, A. R., J. Baddiley, and N. L. Blumson. 1968. The teichoic acids. Advan. Enzymol. **30**:223-253.
17. Archibald, A. R., H. E. Coapes, and G. H. Stafford. 1969. The action of dilute alkali on bacterial cell walls. Biochem. J. **113**:899-900.
18. Archibald, A. R., J. Baddiley, and J. Goundry. 1970. The action of dilute alkali on the glycyl residues of staphylococcal peptidoglycan. Biochem. J. **116**:313-315.
19. Archibald, A. R., J. Baddiley, J. E. Heckels, and S. Heptinstall. 1971. Further studies on the glycerol teichoic acid of walls of *Staphylococcus lactis* I3. Location of phosphodiester groups and their susceptibility to hydrolysis with alkali. Biochem. J. **125**:353-359.
19a. Arima, K., T. Nakamura, and G. Tamura. 1968. Chemical structure of the mucopeptide of *Streptomyces roseochromogenes* cell wall. Agr. Biol. Chem. **32**:530-531.
20. Armstrong, J. J., J. Baddiley, and J. G. Buchanan. 1960. Structure of the ribitol teichoic acid from the walls of *Bacillus subtilis*. Biochem. J. **76**:610-621.
21. Armstrong, J. J., J. Baddiley, and J. G. Buchanan. 1961. Further studies on the teichoic acid from *Bacillus subtilis* walls. Biochem. J. **80**:254-261.
22. Asselineau, J. 1966. The bacterial lipids. Herman and Holden-Day, Paris.
23. Attali, P., and J. Orfila. 1967. Etudes des amino-acides de la paroi de quelques corynebacteries et de *Listeria monocytogenes*. Ann. Inst. Pasteur. **113**:264-266.
24. Auletta, A. E., and E. R. Kennedy. 1966. Deoxyribonucleic acid base composition of some members of the *Micrococcaceae*. J. Bacteriol. **92**:28-34.
25. Azuma, I., D. W. Thomas, A. Adam, J. M. Ghuysen, R. Bonaly, J. F. Petit, and E. Lederer. 1970. Occurrence of N-glycolylmuramic acid in bacterial cell walls. A preliminary survey. Biochim. Biophys. Acta **208**:444-451.
26. Baboolal, R. 1969. Cell wall analysis of oral filament bacteria. J. Gen. Microbiol. **58**:217-226.
27. Baddiley, J., 1968. Teichoic acids and the molecular structure of bacterial cell walls. Proc. Roy. Soc. Ser. B **170**:331-348.
28. Baddiley, J., J. G. Buchanan, F. E. Hardy, R. O. Martin, U. L. Rajbhandary, and A. R. Sanderson. 1961. The structure of the ribitol teichoic acid of *Staphylococcus aureus* H. Biochim. Biophys. Acta **52**:406-407.
29. Baddiley, J., J. G. Buchanan, U. L. Rajbhandary, and A. R. Sanderson. 1962. Teichoic acid from the walls of *Staphylococcus aureus* H. Structure of the N-acetylglucosaminylribitol residues. Biochem. J. **82**:439-448.
30. Bahn, A. N., P. C. Y. Kung, and J. A. Hayashi, 1966. Chemical composition and serological analysis of the cell wall of *Peptostreptococcus*. J. Bacteriol. **91**:1672-1676.
31. Baird-Parker, A. C. 1965. The classification of staphylococci and micrococci from world-wide sources. J. Gen. Microbiol. **38**:363-385.
32. Baird-Parker, A. C., 1970. The relationship of cell wall composition to the current classification of staphylococci and micrococci. Int. J. Sys. Bacteriol. **20**:483-490.
33. Barksdale, L. 1970. *Corynbacterium diphtheriae* and its relatives. Bacteriol. Rev. **34**:378-422.
34. Becker, B., M. P. Lechevalier, and H. A. Lechevalier. 1965. Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. Appl. Microbiol. **13**:236-243.
35. Becker, B., E. M. Wortzel, and J. H. Nelson, III. 1967. Chemical composition of the cell wall of *Caryophanon latum*. Nature (London) **213**:300.
36. Bergan, T., K. Bøvre, and B. Hovig. 1970. A contribution to the taxonomy of *Micrococcaceae*. Publ. Fac. Sci. Univ. J.E. Purkyne Brno **47**:173-177.
37. Bergan, T., K. Bøvre, and B. Hovig. 1970. Present status of the species *Micrococcus freudenreichii* Guillebeau 1891. Int. J. Sys. Bacteriol. **20**:249-254.
38. Bleiweis, A. S., W. W. Karakawa, and R. M. Krause. 1964. Improved technique for the preparation of streptococcal cell walls. J. Bacteriol. **88**:1198-1200.
39. Bogdanovsky, D., E. Interschick-Niebler, K. H. Schleifer, F. Fiedler, and O. Kandler. 1971. γ-Glutamyl-glutamic acid, an interpeptide bridge in the murein of some micrococci and arthrobacter. Eur. J. Biochem. **22**:173-178.
40. Bohacek, J., M. Kocur, and T. Martinec. 1965. Deoxyribonucleic acid base composition and taxonomy of the genus *Micrococcus*. Pub. Fac. Sci. Univ. J. E. Purkyne Brno **35**:318-322.
41. Bohacek, J., M. Kocur, and T. Martinec. 1967. DNA base composition and taxonomy of some

micrococci. J. Gen. Microbiol. **46**:369–376.
42. Bohacek, J., M. Kocur, and T. Martinec. 1968. Deoxyribonucleic acid base composition of some marine and halophilic micrococci. J. Appl. Bacteriol. **31**:215–219.
43. Bohacek, J., M. Kocur, and T. Martinec. 1968. Deoxyribonucleic acid base composition of *Sporosarcina ureae*, Arch. Mikrobiol. **64**:23–28.
44. Bohacek, J., G. Blazicek, M. Kocur, O. Solberg, and O. G. Clausen. 1969. Deoxyribonucleic acid base composition of pediococci and aerococci. Arch. Mikrobiol. **67**:58–61.
45. Boone, C. J., and L. Pine. 1968. Rapid method for characterization of *Actinomycetes* by cell wall composition. Appl. Microbiol. **16**:279–284.
46. Bradley, S. G., and D. Ritzi. 1968. Composition and ultrastructure of *Streptomyces venezuela*. J. Bacteriol. **95**:2358–2364.
47. Braunitzer, G. 1955. Bestimmung der Reihenfolge der Amino-säuren am Carboxylende des Tabakmosaikvirus durch Hydrazinspaltung. Chem. Ber. **88**:2025–2036.
48. Braun, V., K. Rehn, and H. Wolff. 1970. Supramolecular structure of the rigid layer of the cell wall of *Salmonella*, *Serratia*, *Proteus*, and *Pseudomonas fluorescens*. Number of lipoprotein molecules in a membrane layer. Biochemistry **9**:5041–5049.
49. Breed, R. S., E. G. D. Murray, and N. R. Smith. 1957. Bergey's manual of determinative bacteriology. 7th ed. The Williams & Wilkins Co., Baltimore.
50. Bricas, E., J. M. Ghuysen, and Ph. Dezélée. 1967. The cell wall peptidoglycan of Bacillus megaterium KM. I. Studies on the stereochemistry of α-, α'-diaminopimelic acid. Biochemistry **6**:2598–2607.
51. Brooks, D., and J. Baddiley. 1969. The mechanism of biosynthesis and direction of chain extension of a poly-(N-acetyl-glucosamine 1-phosphate) from the walls of *Staphylococcus lactis* N.C.T.C. 2102. Biochem. J. **113**:635–642.
52. Browder, H. P., P. A. Tavormina, and W. A. Zygmunt. 1968. Optical configuration of staphylococcal cell wall serine. J. Bacteriol. **96**:1452–1453.
53. Brundish, D. E., and J. Baddiley. 1967. The characterization of pneumococcal C-polysaccharide as a ribitol teichoic acid. Biochem. J. **105**:30c–31c.
54. Brundish, D. E., and J. Baddiley. 1968. Pneumococcal C-substance, a ribitol teichoic acid containing choline phosphate. Biochem. J. **110**:573–582.
55. Bruneteau, M., and G. Michel. 1968. Structure d'un dimycolate d'arabinose isole de *Mycobacterium marianum*. Chem. Phys. Lipids **2**:229–239.
56. Buchanan, B. B., and L. Pine. 1962. Characterization of a propionic acid producing actinomycete, *Actinomyces propionicus*, sp. nov. J. Gen. Microbiol. **28**:305–323.
57. Buckmire, F. L. A. 1970. The physical structure of the cell wall as a differential character. Int. J. Sys. Bacteriol. **20**:345–360.
58. Bumsted, R. M., J. L. Dahl, D. Söll, and J. L. Strominger. 1968. Biosynthesis of the peptidoglycan of bacterial cell walls. X. Further study of the glycyl transfer ribonucleic acids active in peptidoglycan synthesis in *Staphylococcus aureus*. J. Biol. Chem. **243**:779–783.
59. Campbell, J. N., M. Leyh-Bouille, and J. M. Ghuysen. 1969. Characterization of the *Micrococcus lysodeikticus* type of peptidoglycan in walls of other *Micrococcaceae*. Biochemistry **8**:193–200.
60. Canale-Parola, E. 1970. Biology of sugar-fermenting sarcinae. Bacteriol. Rev. **34**:82–97.
61. Cato, E. P., C. S. Cummins, and L. Ds. Smith. 1970. *Clostridium limosum* André in Prevot 1948, 165 amended description and pathogenic characteristics. Int. J. Syst. Bacteriol. **20**:305–316.
62. Chatterjee, A. N. 1969. Use of bacteriophage-resistant mutants to study the nature of the bacteriophage receptor site of *Staphylococcus aureus*. J. Bacteriol **98**:519–527.
63. Clarke, K., G. W. Gray, and D. A. Reaveley, 1967. The extraction of cell walls of *Pseudomonas aeruginosa* with aqueous phenol. The insoluble residue and material from the aqueous layers. Biochem. J. **105**:759–765.
63a. Collins-Thompson, D. L., T. Sørhaug, L. D. Witter, and Z. J. Ordal. 1972. Taxonomic consideration of *Microbacterium lacticum*, *Microbacterium flavum*, and *Microbacterium thermosphactum*. Int. J. Syst. Bacteriol. **22**: 65–72.
64. Conn, H. J., and J. Dimmick. 1947. Soil bacteria similar in morphology to *Mycobacterium* and *Corynebacterium*. J. Bacteriol. **54**:291–303.
65. Coyette, J., and J. M. Ghuysen. 1970. Structure of the walls of *Lactobacillus acidophilus* strain 63 AM Gasser. Biochemistry **9**:2935–2943.
66. Crum, E. M., and D. J. Siehr. 1967. *Thiobacillus thiooxidans* cell wall amino acids and monosaccharides. J. Bacteriol. **94**:2069–2070.
67. Cummins, C. S. 1954. Some observations on the nature of the antigens in the cell wall of *Corynebacterium diphtheriae*. Brit. J. Exp. Pathol. **35**:166–180.
68. Cummins, C. S. 1962. La composition chimique des parois cellulaires d'actinomycetes et son application taxonomique. Ann. Inst. Pasteur **103**:385–391.
69. Cummins, C. S. 1962. Chemical composition and antigenic structure of the cell walls of *Corynebacterium*, *Mycobacterium*, *Nocardia*, *Actinomyces* and *Arthrobacterium*. J. Gen. Microbiol. **28**:35–50.
70. Cummins, C. S. 1965. Chemical and antigenic studies on cell walls of mycobacteria, corynebacteria and nocardias. Amer. Rev. Resp. Dis. **92**:63–72.
71. Cummins, C. S. 1965. Ornithine in mucopeptide of Gram-positive cell walls. Nature (London) **206**:1272.

72. Cummins, C. S. 1969. *Actinomyces israelii* type 2. *In* H. Prauser (ed.), The *Actinomycetales*. G. Fischer Verlag, Jena.
73. Cummins, C. S. 1970. Cell wall composition in the classification of Gram positive anaerobes. Int. J. Syst. Bacteriol. **20**:413-419.
74. Cummins, C. S. 1971. Cell wall composition in *Corynebacterium bovis* and some other corynebacteria. J. Bacteriol. **105**:1227-1228.
75. Cummins, C. S., and H. Harris. 1956. The chemical composition of the cell wall in some gram-positive bacteria and its possible value as a taxonomic character. J. Gen. Microbiol. **14**:583-600.
76. Cummins, C. S., and H. Harris. 1958. Studies on the cell wall composition and taxonomy of actinomycetales and related groups. J. Gen. Microbiol. **18**:173-189.
77. Cummins, C. S., and J. L. Johnson. 1971. Taxonomy of the clostridia; wall composition and DNA homologies in *Clostridium butyricum* and other butyric-acid-producing clostridia. J. Gen. Microbiol. **67**:33-46.
78. Cummins, C. S., G. Atfield, R. J. W. Rees, and R. C. Valentine. 1967. Cell wall composition in *Mycobacterium lepraemurium* J. Gen. Microbiol. **49**:377-384.
79. Curtis, S. N., and R. M. Krause. 1964. Immunochemical studies on the specific carbohydrate of Group G streptococci. J. Exp. Med. **119**:997-1004.
80. Curtis, S. N., and R. M. Krause. 1964. The identification of rhamnose as an antigenic determinant of group B streptococci carbohydrates. Fed. Proc. **23**:191.
81. Cziharz, B., K. H. Schleifer, and O. Kandler. 1971. A new type of peptide subunit in the murein of *Arthrobacter* strain J39. Biochemistry **10**:3574-3578.
82. DaSilva, G. A. N., and J. G. Holt. 1965. Numerical taxonomy of certain coryneform bacteria. J. Bacteriol. **90**:921-927.
83. Davis, G. H. G., and J. H. Freer. 1960. Studies upon an oral aerobic actinomycete. J. Gen. Microbiol. **23**:163-178.
84. Davis, G. H. G., L. Fomin, E. K. G. Wilson, and K. G. Newton. 1969. Numerical taxonomy of *Listeria*, streptococci and possibly related bacteria. J. Gen. Microbiol. **57**:333-348.
85. Davison, A. L., and J. Baddiley. 1963. The distribution of teichoic acids in staphylococci, J. Gen. Microbiol. **32**:271-276.
86. Davison, A. L., and J. Baddiley. 1964. Glycerol teichoic acids in walls of *Staphylococcus epidermidis*. Nature (London) **202**:874.
87. Deibel, R. H. 1964. The group D streptococci. Bacteriol. Rev. **28**:330-366.
88. Deibel, R. H., and C. F. Niven, Jr. 1960. Comparative study of *Gaffkya homari*, *Aerococcus viridans*, tetrad-forming cocci from meat curing brines, and the genus *Pediococcus*. J. Bacteriol. **79**:175-180.
89. De Petris, S. 1967. Ultrastructure of the cell wall of *Escherichia coli* and chemical nature of its constituent layer. J. Ultrastructr. Res. **19**: 45-83.
90. De Vries, W. S., J. Gerbrandy, and A. H. Stouthamer. 1967. Carbohydrate metabolism in *Bifidobacterium bifidum*. Biochim. Biophys. Acta **136**:415-425.
91. De Weese, M. S., M. J. Slack. 1968. Quantitative analysis of *Actinomyces* cell walls. Appl. Microbiol. **16**:1713-1718.
92. Diringer, H. 1968. Über die Bindung der D-Glutaminsäure im Murein von *E. coli*. Z. Naturforsch. **23b**:883-884.
93. Diringer, H., and D. Jusic. 1966. Über die Bindung der meso-Diaminopimelinsäure im Murein von *E. coli*. Z. Naturforsch. **21b**:603-604.
94. Douglas, L. J., and J. Baddiley. 1968. A lipid intermediate in the biosynthesis of a teichoic acid. Fed. Eur. Biochem. Soc. Lett. **1**:114-116.
95. Douglas, H. C., and S. E. Gunter. 1946. The taxonomic position of *Corynebacterium acnes*. J. Bacteriol. **52**:15-23.
96. Drews, G., and H. Meyer. 1964. Untersuchungen zum chemischen Aufbau der Zellwände von *Anacystis nidulans* and *Chlorogloea fritschii*. Arch. Mikrobiol. **48**:259-267.
97. Elliot, S. D., J. Hayward, and T. Y. Liu. 1971. The presence of a group A variant like antigen in streptococci of other groups with special reference to group N. J. Exp. Med. **133**:479-493.
98. Ellwood, D. C. 1971. The anionic polymers in the cell wall of *Bacillus subtilis* var. *niger* grown in phosphorus-limiting environments supplemented with increasing concentrations of sodium chloride. Biochem. J. **121**:349-351.
99. Ellwood, D. C., and D. W. Tempest. 1969. Control of teichoic acid and teichuronic acid biosynthesis in chemostat cultures of *Bacillus subtilis* var. *niger*. Biochem. J. **111**:1-5.
99a. Ellwood, D. C., and D. W. Tempest. 1972. Effects of environment on bacterial cell wall content and composition. Advan. Microbiol. Physiol. **7**:83-117.
100. Eschenbecher, F. 1969. Zur Kenntnis der biersäuernden Laktobazillen. Brauwissenschaft **22**:14-28.
100a. Evans, J. B., and L. M. Schultes. 1969. DNA base composition and physiological characteristics of the genus *Aerococcus*. Int. J. Syst. Bacteriol. **19**:159-163.
101. Fiedler, F., K. H. Schleifer, B. Cziharz, E. Interschick, and O. Kandler. 1970. Murein types in *Arthrobacter*, *Brevibacteria*, *Corynebacteria*, and *Microbacteria*. Publ. Fac. Sci. Univ. J. E. Purkyne Brno **47**:111-122.
101a. Forrester, I. T., and A. J. Wicken. 1966. The chemical composition of the cell walls of some thermophilic bacilli. J. Gen. Microbiol. **42**:147-154.
102. Frank, H., M. Lefort, and H. H. Martin. 1962. Elektronenoptischeund chemische Untersuchungen an Zellwänden der Blaualge *Phormidium uncinatum*. Z. Naturforsch. **17b**: 262-268.
103. Gasser, F. 1970. Electrophoretic characteriza-

tion of lactic dehydrogenases in the genus *Lactobacillus*. J. Gen. Microbiol. **62**:233–239.
104. Garvie, E. I. 1960. The genus *Leuconostoc* and its nomenclature. J. Dairy Res. **27**:283–292.
105. Garvie, E. I. 1967. *Leuconostoc oenos* sp. nov. J. Gen. Microbiol. **48**:431–438.
106. Garvie, E. I. 1967. The growth factor and amino acid requirements of species of the genus *Leuconostoc*, including *Leuconostoc paramesenteroides* (sp.nov.) and *Lc. oenos*. J. Gen. Microbiol. **48**:439–447.
107. Georg, L. K., and J. H. Brown. 1967. *Rothia*, gen.nov. and aerobic genus of the family *Actinomycetales*. Int. J. Syst. Bacteriol. **17**:79–88.
108. Georg, L. K., G. W. Robertstad, and S. A. Brinkman. 1965. Identification of species of *Actinomycetales*. J. Bacteriol. **88**:477–490.
109. Ghuysen, J. M. 1968. Use of bacteriolytic enzymes in determination of wall structure and their role in cell metabolism. Bacteriol. Rev. **32**:425–464.
110. Ghuysen, J. M., and J. L. Strominger. 1963. Structure of the cell wall of *Staphylococcus aureus*, strain Copenhagen. I. Preparation of fragments by enzymatic hydrolysis. Biochemistry **2**:1110–1119.
111. Ghuysen, J. M., and J. L. Strominger. 1963. Structure of the cell wall of *Staphylococcus aureus*, strain Copenhagen. II. Separation and structure of disaccharides. Biochemistry **2**:1119–1125.
112. Ghuysen, J. M., D. J. Tipper, and J. L. Strominger. 1965. Structure of the cell wall of *Staphylococcus aureus*, strain Copenhagen. IV. The teichoic acid-glycopeptide complex. Biochemistry **4**:474–485.
113. Ghuysen, J. M., D. J. Tipper, C. H. Birge, and J. L. Strominger. 1965. Structure of the cell wall of *Staphylococcus aureus*, strain Copenhagen. VI. The soluble glycopeptide and its sequential degradation by peptidases. Biochemistry **4**:2244–2254.
114. Ghuysen, J. M., D. J. Tipper, and J. L. Strominger. 1966. Enzymes that degrade bacterial cell walls, p. 685–699. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology. Academic Press Inc., New York.
115. Ghuysen, J. M., E. Bricas, M. Leyh-Bouille, M. Lache, and G. D. Shockman. 1967. The peptide N^{α}-(L-alanyl-D-isoglutaminyl)-N^{ϵ}-(D-isoglutaminyl)-N-(D-isoasparaginyl)-L-lysyl-D-alanine and the disaccharide N-acetylglucosaminyl-β-1,4-N-acetylmuramic acid in cell wall peptidoglycan of *Streptococcus faecalis* strain ATCC 9790. Biochemistry **6**:2607–2619.
116. Ghuysen, J. M., E. Bricas, M. Lache, and M. Leyh-Bouille, 1968. Structure of the cell walls of *Micrococcus lysodeikticus*. III. Isolation of a new peptide dimer, N^{α}-[L-alanyl-γ(α-D-glutamyl-glycine)]-L-lysyl-D-alanyl-N^{α}-L-alanyl-γ-(α-D-glutamyl-glycine)]-L-lysyl-D-alanine. Biochemistry **7**:1450–1460.
117. Ghuysen, J. M., J. L. Strominger, and D. J. Tipper. 1968. Bacterial cell walls, p. 53–104. *In* M. Florkin and E. H. Stotz (ed.). Comprehensive biochemistry, vol. 26A. American Elsevier Publishing Co., New York.
118. Glauert, A. M., and M. J. Thornley. 1969. The topography of the bacterial cell wall. Annu. Rev. Microbiol. **23**:159–198.
119. Gledhill, W. E., and L. E. Casida, Jr. 1969. Predominant catalase negative soil bacteria. III. *Agromyces*, gen. nov., microorganisms intermediary to *Actinomyces* and *Nocardia*. Appl. Microbiol. **18**:340–349.
120. Gmeiner, J., O. Lüderitz, and O. Westphal. 1969. Biochemical studies on lipopolysaccharides of *Salmonella* R mutants. 6. Investigations on the structure of the lipid A component. Eur. J. Biochem. **7**:370–379.
121. Goodfellow, M. 1967. Numerical taxonomy of some named bacteria cultures. Can. J. Microbiol. **13**:1365–1374.
122. Gooder, H. 1970. Cell wall composition in the classification of streptococci. Int. J. Syst. Bacteriol. **20**:475–482.
123. Gordon, D. F., Jr. 1967. Reisolation of *Staphylococcus salivarius* from the human oral cavity. J. Bacteriol. **94**:1281–1286.
124. Gotschlich, E., and T. Y. Liu. 1967. Structural and immunological studies on the pneumococcal C-polysaccharide. J. Biol. Chem. **242**: 463–470.
125. Graham, R. K., and J. W. May. 1965. Composition of cell walls of some gram-negative cocci. J. Gen. Microbiol. **41**:243–249.
126. Grant, W. D., and A. J. Wicken. 1970. Autolysis of cell walls of *Bacillus stearothermophilus* B 65 and the chemical structure of the peptidoglycan. Biochem. J. **118**:859–868.
127. Gray, G. W., and S. G. Wilkinson. 1965. The effect of ethylene-diaminetetraacetic acid on the cell walls of some gram-negative bacteria. J. Gen. Microbiol. **39**:385–399.
128. Grula, E. A., and K. D. King. 1970. Inhibition of cell division in *Micrococcus lysodeikticus* dis-II. Can. J. Microbiol. **16**:317–324.
129. Guinand, M., J. M. Ghuysen, K. H. Schleifer, and O. Kandler. 1969. The peptidoglycan in walls of *Butyribacterium rettger*. Biochemistry **8**:200–206.
130. Guinand, M., M. J. Vacheron, and G. Michel. 1970. Structure de parois cellulaires des Nocardia. I. Isolement et composition des parois de *Nocardia kirovani*. Fed. Biochem. Soc. Lett. **6**:37–39.
131. Gutierrez, J. 1953. Numbers and characteristics of lactate utilizing organisms in the rumen of cattle. J. Bacteriol. **66**:123–128.
132. Hall, E. A., and K. W. Knox. 1965. Properties of the polysaccharide and mucopeptide components of the cell wall of *Lactobacillus casei*. Biochem. J. **96**:310–318.
133. Hancock, R., and J. T. Park. 1958. Cell wall synthesis by *Staphylococcus aureus* in the presence of chloramphenicol. Nature (London) **181**:1050–1052.

134. Harrington, B. J. 1966. A numerical taxonomical study of some corynebacteria and related organisms. J. Gen. Microbiol. **45**:31-40.
135. Harrison, A. P., Jr., and A. P. Hansen. 1950. A motile lactobacillus from the cecal feces of turkeys. J. Bacteriol. **59**:444-446.
136. Hase, S., and Y. Matsushima. 1971. Structural studies on a glucose containing polysaccharide obtained from *Micrococcus lysodeikticus* cell walls. II. Determination of the linkage points of N-acetyl-mannosaminuronic acid and its configuration. J. Biochem. **69**:559-566.
137. Hayashi, J. A., and S. S. Barkulis. 1959. Studies of streptococcal cell walls. III. The amino acids of the trypsin-treated cell wall. J. Bacteriol. **77**:177-184.
138. Henssen, A., and D. Schäfer. 1971. Emended description of the genus *Pseudonocardia* Henssen and description of a new species *Pseudonocardia spinosa* Schäfer. Int. J. Syst. Bacteriol. **21**:29-34.
139. Herndon, S. A., and K. F. Bott. 1969. Genetic relationship between *Sarcina ureae* and members of the genus *Bacillus*. J. Bacteriol. **97**:6-12.
140. Heymann, H., L. D. Zeleznick, and J. M. Manniello. 1961. On the mucopeptide fraction of streptococcal cell walls. J. Amer. Chem. Soc. **83**:4859-4861.
141. Hill, L. R., 1966. An index to deoxyribonucleic acid base compositions of bacterial species. J. Gen. Microbiol. **44**:419-437.
141a. Hladny, J., K. H. Schleifer, and O. Kandler. 1972. Die Aminosäuresequenz der threoninhaltigen Mureine einiger Streptokokken. Arch. Mikrobiol. **85**:23-38.
142. Hoecht, H., H. H. Martin, and O. Kandler. 1965. Zur Kenntnis der chemischen Zusammensetzung der Zellwand der Blaualgen. Z. Pflanzenphysiol. **53**:39-57.
143. Holdeman, L. V., E. P. Cato, and W. E. C. Moore. 1971. *Clostridium ramosum* (Viliemin) comb. nov.: emended description and proposed neotype strain. Int. J. Syst. Bacteriol. **21**:35-39.
144. Holzapfel, W., and O. Kandler. 1969. Zur Taxonomie der Gattung Lactobacillus Beijerinck. VI. *Lactobacillus coprophilus* subsp. *confusus* nov. subsp., eine neue Unterart der Untergattung *Betabacterium*. Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. 2. **123**:657-666.
145. Holzapfel, W., V. Scardovi, and O. Kandler. 1969. Die Aminosäuresequenz des Ornithin und Lysin enthaltenden Mureins einiger Stämme von *Lactobacillus bifidus* aus dem Pansen. Z. Naturforsch. **24b**:1524-1528.
146. Hughes, R. C. 1967. Composition of the mucopeptide present in the cell walls of *Bacillus licheniformis*. Biochem. J. **102**:26p.
147. Hughes, R. C. 1968. The cell wall of *Bacillus licheniformis* N.C.T.C. 6346. I. Isolation of low molecular weight fragments from the soluble mucopeptide. Biochem. J. **106**:49-59.
148. Hughes, R. C. 1970. Autolysis of isolated cell walls of *Bacillus licheniformis* N.C.T.C. 6346 and *Bacillus subtilis* Marburg strain 168. Separation of the products and characterization of the mucopeptide fragments. Biochem. J. **119**:849-860.
148a. Hughes, R. C. 1971. Autolysis of *Bacillus cereus* cell walls and isolation of structural components. Biochem. J. **121**:791-802.
149. Hughes, R. C., and P. F. Thurman. 1970. Some structural features of the teichuronic acid of *Bacillus licheniformis* N.C.T.C. 6346 cell walls. Biochem. J. **117**:441-449.
150. Hungerer, K. D., and D. J. Tipper. 1969. Cell wall polymers of *Bacillus sphaericus* 9602. I. Structure of the vegetative peptidoglycan. Biochemistry **8**:3577-3587.
151. Hungerer, K. D., J. Fleck, and D. J. Tipper. 1969. Structure of the cell wall peptidoglycan of *Lactobacillus casei* R 094. Biochemistry **8**:3567-3577.
152. Hunter, M. I. S., and D. Thirkell. 1971. Variation in fatty acid composition of *Sarcina flava* membrane lipid with the age of the culture. J. Gen. Microbiol. **65**:115-118.
153. Ikawa, M. 1961. The partial chemical degradation of the cell walls of *Lactobacillus plantarum*, *Streptococcus facealis* and *Lactobacillus casei*. J. Biol. Chem. **236**:1087-1092.
154. Ikawa, M., and E. E. Snell. 1956. D-Glutamic acid and amino sugars as cell wall constituents in lactic acid bacteria. Biochim. Biophys. Acta **19**:576-578.
155. Ikawa, M., and E. E. Snell. 1960. Cell wall composition of lactic acid bacteria. J. Biol. Chem. **235**:1376-1382.
156. Interschick, E., F. Fiedler, K. H. Schleifer, and O. Kandler. 1970 Glycine amide a constituent of the murein of *Arthrobacter atrocyaneus*. Z. Naturforsch. **25**:714-717.
157. Ioneda, T., E. Lederer, and J. Rozanis. 1970. Sur la structure des diesters de tréhalose ("cord factors") produits par *Nocardia asteroides* et *Nocardia rhodochrous*. Chem. Phys. Lipid **4**:375-392.
158. Izaki, K., and J. L. Strominger. 1968. Biosynthesis of the peptidoglycan of bacterial cell walls. XIV. Purification and properties of two D-alanine carboxypeptidases from *Escherichia coli*. J. Biol. Chem. **243**:3139-3201.
159. James, A. M., M. J. Hill, and W. R. Maxted. 1965. A comparative study of bacterial cell wall, protoplast membrane, and L-form envelope of *Streptococcus pyogenes*. Antonie von Leeuwenhoek J. Microbiol. Serol. **31**:423-432.
160. Janczura, E., H. R. Perkins, and H. J. Rogers. 1961. Teichuronic acid: a mucopolysaccharide present in wall preparations from vegetative cells of *Bacillus subtilis*. Biochem. J. **80**:82-93.
161. Jensen, H. L. 1966. Some introductory remarks on the coryneform bacteria. J. Appl. Bacteriol. **29**:13-16.
162. Jeffries, L., M. A. Cawthorne, M. Harris, B. Cook, and A. T. Diplock. 1969. Menaquinone

determination in the taxonomy of *Micrococcaceae*. J. Gen. Microbiol. **54**:365–380.

162a. Johnson, J. L., and C. S. Cummins. 1972. Cell wall composition and deoxyribonucleic acid similarities among the anaerobic coryneforms, classical propionibacteria, and strains of *Arachnia propionica*. J. Bacteriol. **109**:1047–1066.

163. Johnson, K. G., and J. N. Campbell. 1972. Effect of growth conditions on peptidoglycan structure and susceptibility to lytic enzymes in cell walls of *Micrococcus sodonensis*. Biochemistry **11**:277–286.

164. Jones, J. E., and P. Hirsch. 1968. Cell wall composition of *Hyphomicrobium* species. J. Bacteriol. **96**:1037–1041.

165. Jones, L. A., and S. G. Bradley. 1964. Phenetic classification of actinomycetes. Develop. Ind. Microbiol. **5**:267–272.

166. Joseph, R., S. C. Holt, and E. Canale-Parola. 1970. Ultrastructure and chemical composition of the cell wall of *Spirochacta stenostrepta*. Bacteriol. Proc. p. 57.

166a. Jusic, P., C. Roy, and R. W. Watson. 1964. Sequence studies on bacterial cell wall peptides. Can. J. Biochem. **42**:1553–1559.

167. Kandler, O. 1967. Die chemische Zusammensetzung der Bakterienzellwand als chemotaxonomisches Merkmal. Zbl. Bakt. I. Abt. Orig. **205**:198–209.

168. Kandler, O., 1967. Taxonomie und technologische Bedeutung der Gattung *Lactobacillus* Beijerinck. Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. 1 (Suppl.) **2**:139–164.

169. Kandler, O. 1970. Amino acid sequence of the murein and taxonomy of the genera *Lactobacillus*, *Bifidobacterium*, *Leuconostoc* and *Pediococcus*. Int. J. Syst. Bacteriol. **20**:491–507.

170. Kandler, O., and I. G. Abo-Elnaga. 1966. Zur Taxonomie der Gattung *Lactobacillus* Beijerinck, IV. L. *corynoides* ein Synonym von *L. viridescens*, Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. 2 **120**:753–754.

171. Kandler, O. and C. Zehender. 1956. Papierchromatographische Untersuchung der Aminosäurezusammensetzung verschiedener Bakterien-Hydrolysate. Arch. Mikrobiol. **24**:41–48.

172. Kandler, O., A. Hund, and C. Zehender. 1958. Eine einfache Methode zur Isolierung der "Membran-Grundsubstanz" grampositiver and gramnegativer Bakterien. Arch. Mikrobiol. **30**:355–362.

173. Kandler, O., A. Hund, and W. Kundrat. 1959. Die Bedeutung der Aminosäurezusammensetzung der Bakterienmembran für die Differenzierung milchwirtschaftlich wichtiger Bakterien. Proc. XV. Intern. Dairy Congr. **3**: 1369–1375.

174. Kandler, O., R. Plapp, and W. Holzapfel. 1967. Die Aminosäuresequenz des serinhaltigen Mureins von *Lactobacillus viridescens* und *Leuconostoc*. Biochim. Biophys. Acta **147**:252–261.

175. Kandler, O., K. H. Schleifer, and R. Dandl. 1968. Differentation of *Streptococcus faecalis* Andrews and Horder and *Streptococcus faecium* Orla-Jensen based on the amino acid composition of their murein. J. Bacteriol. **96**:1935–1939.

176. Kandler, O., D. Koch, and K. H. Schleifer. 1968. Die Aminosäuresequenz eines glycinhaltigen Mureins einiger Stämme von *Lactobacillus bifidus*. Arch. Mikrobiol. **61**:181–186.

177. Kandler, O., K. H. Schleifer, E. Niebler, M. Nakel, H. Zahradnik, and M. Ried. 1970. Murein types in micrococci and similar organisms. Publ. Fac. Sci. Univ. J. E. Purkyne Brno **47**:143–156.

178. Kandler, O., D. Claus, and A. Moore. 1972. Die Aminosäuresequenz des Mureins von *Sarcina ventriculi* and *Sarcina maxima*. Arch. Mikrobiol. **82**:140–146.

179. Kane, J., H. Lackland, W. W. Karakawa, and R. M. Krause. 1969. Chemical studies on the structure of mucopeptide isolated from *Streptococcus bovis*. J. Bacteriol. **99**:175–179.

180. Kanetsuna, F. 1968. Chemical analysis of mycobacterial cell walls. Biochim. Biophys. Acta **158**:130–143.

181. Kanetsuna, F., T. Imaeda, and F. San Blas. 1968. Chemical analysis of the cell wall of murine leprosy bacillus. J. Bacteriol. **96**:860–861.

182. Kanetsuna, F., T. Imaeda, and G. Cunto. 1969. On the linkage between mycolic acid and arabinogalactan in phenol-treated mycobacterial cell walls. Biochim. Biophys. Acta **173**:341–344.

183. Kanetsuna, F., and G. San Blas. 1970. Chemical analysis of a mycolic acid-arabinogalactan-mucopeptide complex of mycobacteria cell wall. Biochim. Biophys. Acta **208**:434–443.

184. Karakawa, W. W., and R. M. Krause. 1966. Studies on the immunochemistry of streptococcal mucopeptide. J. Expt. Med. **124**: 155–171.

185. Karakawa, W. W., H. Lackland, and R. M. Krause. 1967. Antigenic properties of the hexosamine polymer of streptococcal mucopeptide. J. Immunol. **99**:1178–1182.

186. Karakawa, W. W., J. E. Wagner, and J. H. Pazur. 1971. Immunochemistry of the cell wall carbohydrate of group L hemolytic streptococci. J. Immunol. **107**:554–562.

187. Kates, M. 1964. Bacterial lipids. Advan. Lipid Res. **2**:17–90.

188. Kato, K., J. L. Strominger, and S. Kotani. 1968. Structure of the cell wall of *Corynebacterium diphteriae*. I. Mechanism of hydrolysis by the L-3 enzyme and the structure of the peptide. Biochemistry **7**:2762–2773.

189. Katz, W., M. Matsuhashi, C. P. Dietrich, and J. L. Strominger. 1967. Biosynthesis of the peptidoglycans of bacterial cell walls. IV. Incorporation of glycine in *Micrococcus lysodeikticus*.

J. Biol. Chem. **242**:3207-3217.
190. Katz, W., and H. H. Martin. 1970. Peptide cross-linkage in cell wall murein of *Proteus mirabilis* and its penicillin induced unstable L-form. Biochem. Biophys. Res. Commun. **39**:744-749.
191. Kauffmann, F. 1966. The bacteriology of the *Enterobacteriaceae* The Williams & Wilkins Co., Baltimore.
192. Key, B. A., G. W. Gray, and S. G. Wilkinson. 1970. The effect of ethylene-diaminetetraacetate on *Pseudomonas alcaligenes* and the composition of the bacterial cell wall. Biochem. J. **117**:721-732.
193. Kingan, S. L., and J. E. Ensign. 1968. Chemical composition of the cell wall of *Bacillus thuringiensis* var. *thuringiensis*. J. Bacteriol. **95**:724-726.
194. Kitahara, K., and J. Suzuki. 1963. *Sporolactobacillus* nov. subgen. J. Gen. Appl. Microbiol. **9**:59-71.
195. Kloos, W. E. 1969. Transformation of *Micrococcus lysodeikticus* by various members of the family *Micrococcaceae*. J. Gen. Microbiol. **59**:247-255.
196. Knox, K. W., and E. A. Hall. 1964. The isolation of oligosaccharides from the cell wall polysaccharide of *Lactobacillus casei* serological group C. Biochem. J. **94**:525-533.
197. Knox, K. W., and A. J. Wicken. 1970. Serological properties of the cell wall and membrane teichoic acids from *Lactobacillus helveticus* NCIB8025. J. Gen. Microbiol. **63**:237-248.
198. Knüsel, F., J. Nuesch, M. Scherrer, and K. Schmid. 1967. Einfluss von Lanthionin auf das Wachstum einer Diaminopimelinsäure-heterotrophen Mutante von *Escherichia coli* Pathol. Microbiol. (Basel) **30**:871-879.
199. Koch, D., K. H. Schleifer, and O. Kandler. 1970. Die Aminosäuresequenz des Serin und Asparaginsäure enthaltenden Mureins von *Bifidobacterium bifidum* Orla Jensen. Z. Naturforsch. **25b**:1294-1301.
200. Koch, D., K. H. Schleifer, and O. Kandler. 1970. Die Aminosäuresequenz des Threonin und Serin enthaltenden Murei von *Bifidobacterium longum* Reuter. Arch. Mikrobiol. **74**: 315-325.
201. Kocur, M., and Z. Pacova. 1970. The taxonomic status of *Micrococcus roseus* Flügge 1886. Int. J. Syst. Bacteriol. **20**:233-240.
202. Kocur, M., Z. Pacova, W. Hodgkiss, and T. Martinec. 1970. The taxonomic status of the genus *Planococcus* Migula 1894. Int. J. Syst. Bacteriol. **20**:241-248.
203. Kocur, M., T. Bergan, and N. Mortensen. 1971. DNA base composition of gram-positive cocci. J. Gen. Microbiol. **69**:167-183.
204. Kolenbrander, P. E., and J. C. Ensign. 1968. Isolation and chemical structure of the peptidoglycan of *Spirillum serpens* cell walls. J. Bacteriol. **95**:201-210.
205. Korman, R. Z. 1966. Elevated cell wall serine in pleiotropic staphylococcal mutant. J. Bacteriol. **92**:762-768.
206. Kotani, S., I. Yanagida, K. Kato, and T. Matsuda. 1970. Studies on peptides, glycopeptides and antigenic polysaccharide-glycopeptide complexes isolated from an L-11 enzyme lysate of the cell walls of *Micobacterium tuberculosis* strain H 37 RV. Biken J. **13**:249-275.
207. Krause, R. M. 1963. Symposium on relationship of structure of microorganisms to their immunological properties. IV. Antigenic and biochemical composition of hemolytic streptococcal cell walls. Bacteriol. Rev. **27**:369-380.
208. Krause, R. M., and M. McCarty. 1961. Studies on the chemical structure of the streptococcal cell wall. I. The identification of a mucopeptide in the cell walls of groups A and A variant streptococci. J. Expt. Med. **114**:127-140.
209. Krause, R. M., and M. McCarty. 1962. Studies on the chemical structure of streptococcal cell wall. II. The composition of group C cell walls and chemical basis for serological specificity of the carbohydrate moiety. J. Expt. Med. **115**:49-62.
210. Krulwich, T. A., J. C. Ensign, D. J. Tipper, and J. L. Strominger. 1967. Sphere-rod morphogenesis in *Arthrobacter crystallopoietes*. I. Cell wall composition and polysaccharides of the peptidoglycan. J. Bacteriol. **94**:734-740.
211. Krulwich, T. A., J. C. Ensign, D. J. Tipper, and J. L. Strominger. 1967. Sphere-rod morphogenesis in *Arthrobacter crystallopoietes*. II. Peptides of the cell wall peptidoglycan. J. Bacteriol. **94**:741-750.
212. Lacey, M. S. 1955. The cytology and relationship of *C. fascians* Brit. Mycol. Soc. Trans. **38**:49-58.
213. Lancefield, R. C. 1940/41. Specific relationship of cell composition to biological activity of hemolytic streptococci. Harvey Lect. **36**: 251-290.
214. Lanéelle, M. A., and J. Asselineau. 1970. Characterisation de glycolipides dans une souche de *Nocardia braziliensis*. Fed. Eur. Biochem. Soc. Lett. **7**:64-67.
215. Larsen, H. 1969. Extremely halophilic bacteria. J. Gen. Microbiol. **55**:XXII.
216. Larsen, D. H., D. C. Snetsinger, and P. E. Waibel. 1971. Procedure for determination of D-amino acids. Anal. Biochem. **39**:395-401.
217. Lechevalier, H. A., and M. P. Lechevalier. 1967. Biology of *Actinomycetes*, Annu. Rev. Microbiol. **21**:71-100.
218. Lechevalier, H. A., and M. P. Lechevalier. 1970. A critical evaluation of genera of aerobic actinomycetes. *In* H. Prauser (ed.), The *Actinomycetales*. Gustav Fischer Verlag, Jena.
219. Lechevalier, M. P., and H. A. Lechevalier. 1970. Chemical composition as a criterion in the classification aerobic actinomycetes. Int. J. Syst. Bacteriol. **20**:435-443.
220. Leyh-Bouille, M., J. M. Ghuysen, D. J. Tipper, and J. L. Strominger 1966. Structure of the cell wall of *Micrococcus lysodeikticus*. I. Study of the structure of the glycan. Biochemistry **5**:3079-3090.
221. Leyh-Bouille, M., R. Bonaly, J. M. Ghuysen, R.

Tinelli, and D. J. Tipper. 1970. L,L-diaminopimelic acid containing peptidoglycans in walls of *Streptomyces spec.* and *Clostridium perfringens* (type A). Biochemistry **9**:2944-2951.

222. Linder, R., and S. C. Holt. 1970. Cytology and chemistry of the cell wall of *Bacillus macroides* strains A and P. Bacteriol. Proc., p. 73.
223. Liu, T. Y., and E. C. Gotschlich. 1963. The chemical composition of pneumococcal C-polysaccharide. J. Biol. Chem. **238**:1928-1934.
224. Liu, T. Y., and E. C. Gotschlich. 1967. Muramic acid phosphate as a component of the mucopeptide of gram-positive bacteria. J. Biol. Chem. **242**:471-476.
225. Luedemann, G. M. 1968. *Geodermatophilus*, a new genus of the *Dermatophilaceae* (*Actinomycetales*). J. Bacteriol. **96**:1848-1858.
226. Lüderitz, O., F. Kauffmann, H. Stierlin, and O. Westphal. 1960. Zur Immunchemie der O-Antigene von *Enterobacteriaceae* II. Vergleich der Zuckerbausteine von *Salmonella* S-, T- und R-Formen. Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. 1, Orig. **179**:180-186.
227. Lüderitz, O., M. Staub, and O. Westphal. 1966. Immunochemistry of O and R antigens of *Salmonella* and related *Enterobacteriaceae*. Bacteriol. Rev. **20**:192-255.
228. Lüderitz, O., K. Jann, and R. Wheat. 1968. Somatic and capsular antigens of gram-negative bacteria. *In* M. Florkin and E. H. Stotz (ed.), Comprehensive biochemistry, vol. 26A. American Elsevier Publishing Co., New York.
229. Malik, A. C., G. W. Reinbold, and E. R. Vedamuthu. 1968. An evaluation of the taxonomy of *Propionibacterium*. Can. J. Microbiol. **14**:1185-1191.
230. Manasse, R. J., and W. A. Corpe. 1967. Chemical composition of cell envelopes from *Agrobacterium tumefaciens* Can. J. Microbiol. **41**: 243-249.
231. Mandelstam, J. 1961. Isolation of lysozymesoluble mucopeptides from the cell walls of *Escherichia coli*. Nature (London) **189**:855-856.
232. Mandelstam, J., and H. J. Rogers. 1959. The incorporation of amino acids into the cell wall mucopeptide of staphylococci and the effect of antibiotics on the process. Biochem. J. **72**:651-662.
233. Mann, S. 1969. Uber die Zellwandbausteine von *Listeria monocytogenes* und *Erysipelothrix rhusiopathiae*. Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. 1, Orig. **209**:510-522.
234. Mardarowicz, C. 1966. Isolierung und Charakterisierung des Murein-Sacculus von *Brucella*. Z. Naturforsch. **21b**:1006-1007.
235. Marr, A. G., and J. L. Ingraham. 1962. Effect of temperature on the composition of fatty acids in *Escherichia coli*. J. Bacteriol. **84**:1260-1267.
236. Martin, H. H. 1964. Composition of the mucopolymer in cell walls of the unstable and stable L-forms of *Proteus mirabilis*. J. Gen. Microbiol. **36**:441-450.
237. Martin, H. H. 1966. Biochemistry of bacterial cell walls. Annu. Rev. Biochem. **35**: (part 2):457-483.
238. Martin, H. H. 1967. Murein structure in cell walls of normal bacteria and L-forms of *Proteus mirabilis* and the site of action of penicillin. Folia Microbiol. **12**:234-239.
239. Mason, D. J., and D. Powelson. 1958. The cell wall of *Myxococcus xanthus*. Biochim. Biophys. Acta **29**:1-7.
240. Matsuhashi, M., C. P. Dietrich, and J. L. Strominger. 1967. The role of soluble RNA and of lipid intermediates in glycine incorporation in *Staphylococcus aureus*. J. Biol. Chem. **242**:3191-3206.
241. Matsuda, T., S. Kotani, and K. Kato. 1968. Structure of the cell walls of *Lactobacillus plantarum* ATCC 8014. I. Isolation and identification of the peptides released from cell wall peptidoglycans by *Streptomyces* L-3-enzyme Biken J. **11**:111-126.
242. Matsuda, T., S. Kotani, and K. Kato. 1968. Structure of the cell walls of *Lactobacillus plantarum* ATCC 8014. II. Cross linkage between D-alanine and α,α'-diaminopimelic acid in the cell wall peptidoglycans studied with an L-11 enzyme from *Flavobacterium sp.* Biken J. **11**:127-138.
243. Matsuda, T., S. Kotani, K. Kato, T. Katayama, T. Moriyama, and M. Yoneda. 1968. Isolation of D-alanyl-meso-α,α'-diaminopimelic acid endopeptidase from a crude preparation in *Streptomyces* "L-3 enzyme". Biken J. **11**:145-148.
244. Mazanec, K., M. Kocur, and T. Martinec. 1966. Electron microscopy of ultrathin sections of *Micrococcus cryophilus*. Can. J. Microbiol. **12**:465-469.
245. Michel, G., C. Bordet, and E. Lederer. 1960. Isolement d'un nouvel acide mycolique l'acide nocardique, d'partir d'un souche *Nocardia asteroides*. C. R. Acad. Sci. **250**:3518-3520.
246. Michel, M. F., and H. Gooder. 1962. Amino acids, amino sugar and sugars present in the cell wall of some strains of *Streptococcus pyogenes*. J. Gen. Microbiol. **29**:199-205.
247. Michel, M. F., and J. M. N. Willers. 1964. Immunochemistry of group F streptococci, isolation of group specific oligosaccharides. J. Gen. Microbiol. **37**:381-389.
248. Michel, M. F., J. van Vonno, and R. M. Krause. 1969. Studies on the chemical structure and the antigenic determinant of type II antigen of the group F streptococci. J. Immunol. **102**:215-221.
249. Miller, I., R. Plapp, and O. Kandler. 1966. The amino acid sequence of the serine containing murein of *Butyribacterium rettgeri*. Biochem. Biophys. Res. Commun. **25**:415-420.
250. Miller, I. R. Plapp, and O. Kandler. 1968. Isolierung und Identifizierung eines Serinhal-

tigen UDP-Muramyl-Tripeptides aus *Butyribacterium rettgeri*. Z. Naturforsch. **23b:** 217-220.
251. Miller, I. L., and S. J. Silverman. 1959. Glucose metabolism of *Listeria monocytogenes*. Bacteriol. Proc., p. 103.
252. Mirelman, D., and N. Sharon. 1966. Isolation and characterization of two disaccharidepeptides from lysozyme digests of *Micrococcus lysodeikticus* cell walls. Biochem. Biophys. Res. Commun. **24:**237-243.
253. Mirelman, D., and N. Sharon. 1967. Isolation and study of the chemical structure of low molecular weight glycopeptides from *Micrococcus lysodeikticus* cell walls. J. Biol. Chem. **242:**3414-3427.
254. Mirelman, D., and N. Sharon. 1968. Isolation and characterization of the disaccharide N - acetylglucosaminyl - β(1 - 4) - N - acetylmuramic acid and two tripeptide derivatives of this disaccharide from lysozyme digests of *Bacillus licheniformis* ATCC 9945 cell walls. J. Biol. Chem. **243:**2279-2288.
255. Misaki, A., S. Yukawa, K. Tschuiya, and T. Yamasaki. 1966. Studies on the cell walls of *Mycobacteria*. I. Chemical and biological properties of the cell walls and mucopeptide of BCG. J. Biochem. **59:**388-396.
256. Misaki, A., N. Ikawa, I. Kato, and S. Kotani. 1970. Cell wall arabinogalactan of *Mycobacterium phlei*. Biochim. Biophys. Acta **215:**405-408.
257. Modarska, H., and M. Modarski. 1969. Comparative studies on the occurrence of lipid A, DAP and arabinose in nocardia cells. Arch. Immun. Ther. Exp. **17:**739-743.
258. Moore, W. E. C. 1970. Relationship of metabolic products to taxonomy of anaerobic bacteria. Int. J. Syst. Bacteriol. **20:**535-538.
259. Moore, W. E. C., and E. P. Cato. 1963. Validity of *Propionibacterium acnes* (Gilchrist) Douglas and Gunder comb. nov. J. Bacteriol. **85:** 870-874.
260. Moore, W. E. C., and E. P. Cato. 1965. Synonymy of *Eubacterium limosum* and *Butyribacterium rettgeri: Butyribacterium limosum* comb. nov. Int. J. Syst. Bacteriol. **16:**69-80.
261. Morrison, S. J., T. G. Tornabene, and W. E. Kloos. 1971. Neutral lipids in the study of relationships of members of the family *Micrococcaceae*. J. Bacteriol. **108:**353-358.
262. Mortensen, N., and M. Kocur. 1967. Correlation of DNA base composition and acid formation from glucose of staphylococci and micrococci. Acta Pathol. Microbiol. Scand. **69:**445-457.
263. Mosser, J. L., and A. Tomasz. 1970. Choline-containing teichoic acid as a structural component of penumococcal cell wall and its role in sensitivity to lysis by an autolytic enzyme. J. Biol. Chem. **245:**287-298.
264. Mulder, E. G., and J. Antheunisse. 1963. Morphologie, physiologie et écologie des *Arthrobacter*. Ann. Inst. Pasteur **105:**46-74.
265. Munoz, E., J. M. Ghuysen, M. Leyh-Bouille, J. F. Petit, and R. Tinelli. 1966. Structural variations in bacterial cell wall peptidoglycans studied with *Streptomyces* F_1 endo-N-acetylmuramidase. Biochemistry **5:**3091-3098.
266. Munoz, E., J. M. Ghuysen, and H. Heymann. 1967. Cell walls of *Streptococcus pyogenes* Type 14. C polysaccharide-peptidoglycan and G polysaccharide-peptidoglycan complexes. Biochemistry **6:**3659-3670.
267. Nakamura, T., G. Tamura, and K. Arima. 1967. Structure of the cell walls of *Streptomyces*. J. Ferment Technol. **45:**869-878.
268. Nakel, M., J. M. Ghuysen, and O. Kandler. 1971. Wall peptidoglycan in *Aerococcus viridans* strains 201 Evans and ATCC 11563 and in *Gaffkya homari* strain ATCC 10400. Biochemistry **10:**2170-2175.
269. Nermut, M. V., and R. G. E. Murray. 1967. Ultrastructure of the cell wall of *Bacillus polymyxa*. J. Bacteriol. **93:**1949-1965.
270. Nguyen-Dan, T. 1969. Sur la composition chimique des parois cellulaires du *Corynebacterium parvum*. C. R. Acad. Sci. 269:1455-1457.
271. Niebler, E., K. H. Schleifer, and O. Kandler. 1969. The amino acid sequence of the L-glutamic acid containing mureins of *Mc. luteus* and *Mc. freudenreichii*. Biochem. Biophys. Res. Commun. **34:**560-568.
272. Nikaido, H. 1970. Lipopolysaccharide in the taxonomy of *Enterobacteriaceae*. Int. J. Syst. Bacteriol. **20:**383-406.
273. Niven, C. F., Jr., and J. B. Evans. 1957. *Lactobacillus viridescens* nov. spec. A heterofermentative species that produces a green discoloration of cured meat pigments. J. Bacteriol. **73:**758-759.
274. Novotny, P. 1969. Composition of cell walls of *Clostridium sordellii* and *Clostridium bifermentans* and its relation to taxonomy. J. Med. Microbiol. **2:**81-100.
275. Oeding, P. 1966. Antigenic structure of staphylococci. Postepy Mikrobiol. **5:**221-230.
276. Onisi, M. 1949. Study on the *Actinomyces* isolated from the deeper layers of various dentine. Shikagaku Zasshi **6:**273-282.
277. Orla-Jensen, S. 1919. The lactic acid bacteria. Memb. Acad. Roy. Sci. Danmark, Sect. Sci. **5:**81-197. Andr. Fred Host and Son, Copenhagen.
278. Orskov, J. 1938. Untersuchungen über Strahlenpilze, reingezüchtet aus dänischen Erdproben. Zentrabl. Bakteriol. Parasitenk. Infektionstr. Hyg. Abt. 2. **98:**344-357.
279. Osborn, M. J. 1969. Structure and biosynthesis of bacterial cell wall. Annu. Rev. Biochem. **38:**501-538.
280. Park, J. T., and R. Hancock. 1960. A fractionation procedure for studies of the synthesis of cell wall mucopeptide and other polymers in cells of *Staphylococcus aureus*. J. Gen. Microbiol. **22:**249-258.
281. Partridge, M. D., A. L. Davison, and J. Baddiley. 1971. A polymer of glucose and N-acetyl-galactosamine-1-phosphate in the

wall of *Micrococcus spec.* A 1. Biochemistry **121**:695–700.

282. Pazur, J. H., A. Cepure, J. A. Kane, and W. W. Karakawa. 1971. Glycans from streptococcal cell walls: Glycosyl-phosphoryl moieties as immunodominant groups in heteroglycans from group D and group L streptococci. Biochem. Biophys. Res. Commun. **43**:1421–1428.
283. Perkins, H. R. 1963. Chemical structure and biosynthesis of bacterial cell walls. Bacteriol. Rev. **27**:18–55.
284. Perkins, H. R. 1963. A polymer containing glucose and aminohexuronic acid isolated from the cell walls of *Micrococcus lysodeikticus*. Biochem. J. **86**:475–483.
285. Perkins, H. R. 1965. 2,6-Diamino-3-hydroxypimelic acid in microbial cell wall mucopeptide. Nature (London) **208**:872–873.
286. Perkins, H. R. 1965. Homoserine in the cell walls of plant pathogenic corynebacteria. Biochem. J. **97**:3c–5c.
287. Perkins, H. R. 1965. The action of hot formamide on bacterial cell walls. Biochem. J. **95**:876–882.
288. Perkins, H. R. 1967. The use of photolysis of dinitrophenyl-peptides in structural studies on the cell-wall mucopeptide of *Corynebacterium poinsettiae*. Biochem. J. **102**:29c–32c.
289. Perkins, H. R. 1968. Cell wall mucopeptide of *Corynebacterium insidiosum* and *Corynebacterium sepedonicum*. Biochem. J. **110**:47p–48p.
290. Perkins, H. R. 1969. The configuration of 2,6-diamino-3-hydroxy-pimelic acid in microbial cell walls. Biochem. J. **115**:797–805.
291. Perkins, H. R. 1971. Homoserine and diaminobutyric acid in the mucopeptide-precursor-nucleotides and cell walls of some plant-pathogenic corynebacteria. Biochem. J. **121**:417–423.
292. Perkins, H. R., and C. S. Cummins. 1964. Ornithine and 2,4-diamino-butyric acid as components of the cell walls of plant pathogenic *Corynebacterium*. Nature (London) **201**:1105–1107.
293. Petit, J. F., E. Munoz, and J. M. Ghuysen. 1966. Peptide cross-links in bacterial cell wall peptidoglycans studied with specific endopeptidases from *Streptomyces albus* G. Biochemistry **5**:2764–2776.
294. Petit, J. F., J. L. Strominger, and D. Söll. 1968. Biosyntheis of the peptidoglycan of bacterial cell walls. VII. Incorporation of serine and glycine into interpeptide bridges in *Staphylococcus epidermidis*. J. Biol. Chem. **243**: 757–768.
295. Petit, J. F., A. Adam, J. Wietzerbin-Falszpan, E. Lederer, and J. M. Ghuysen. 1969. Chemical structure of the cell wall of *Mycobacterium smegmatis*. I. Isolation and partial characterization of the peptidoglycan. Biochem. Biophys. Res. Commun. **35**:478–485.
296. Pickering, B. T. 1966. Components of the cell wall of *Clostridium welchii* (type A). Biochem. J. **100**:430–440.
297. Pine, L. 1970. Classification and phylogenetic relationship of microaerophilic actinomycetes. Int. J. Syst. Bacteriol. **20**:445–474.
298. Pine, L., and C. J. Boone. 1967. Comparative cell wall analyses of morphological forms within the genus *Actinomyces*. J. Bacteriol. **94**:875–883.
299. Pine, L., and L. Georg. 1965. The classification and phytogenetic relationships of *Actinomycetales*. Int. J. Syst. Bacteriol. **15**:143–163.
300. Plapp, R., and O. Kandler. 1965. Zur Wirkungsweise zellwandhemmender Antibiotica bei gramnegativen Bakterien. I. Die Wirkung von Penicillin auf die Konzentration von Zellwandvorstufen bei *Proteus mirabilis*. Arch. Mikrobiol. **50**:171–193.
301. Plapp, R., and O. Kandler. 1967. Isolation of an ornithine-containing cell wall precursor of *Lactobacillus cellobiosus*. Biochem. Biophys. Res. Commun. **28**:141–145.
302. Plapp, R., and O. Kandler. 1967. Identification of L-ornithine and δ-aminosuccinyl ornithine in cell wall hydrolysates of *Lactobacillus cellobiosus*. Nature (London) **213**:803–804.
303. Plapp, R., and O. Kandler. 1967. Die Aminosäuresequenz des Asparaginsäure enthaltenden Mureins von *Lactobacillus coryneformis* und *Lactobacillus cellobiosus*. Z. Naturforsch. **22b**:1062–1067.
304. Plapp, R., K. H. Schleifer, and O. Kandler. 1967. The amino acid sequence of the mureins of lactic acid bacteria. Folia Microbiol. (Prague) **12**:206–213.
305. Plapp, R., and J. L. Strominger. 1970. Biosynthesis of the peptidoglycan of bacterial cell walls. XVII. Biosynthesis of peptidoglycan and of interpeptide bridges in *Lactobacillus viridescens*. J. Biol. Chem. **245**:3667–3674.
306. Powell, J. F., and R. E. Strange. 1957. α, ε-Diaminopimelic acid metabolism and sporulation in *Bacillus sphaericus*. Biochem. J. **65**:700–708.
307. Prauser, H. 1970. State and tendencies in the taxonomy of *Actinomycetales*. *In* H. Prauser (ed.), The *Actinomycetales*. Gustav Fischer Verlag, Jena.
308. Prauser, H., M. P. Lechevalier, and H. Lechevalier. 1970. Description of *Oerskovia* gen.n. to harbor Orskov's motile *Nocardia*. Appl. Microbiol. **19**:534.
309. Prévot, A. R. 1970. Importance of the biochemical composition of the cell walls as a taxonomic characteristic. Int. J. Syst. Bacteriol. **20**:539–540.
310. Primosigh, J., H. Pelzer, D. Maass, and W. Weidel. 1961. Chemical characterization of mucopeptides released from the *E. coli* B cell wall by enzymic action. Biochim. Biophys. Acta **46**:68–80.
311. Ramamurthi, C. S. 1959. Comparative studies

on some gram-positive phytopathogenic bacteria and their relationship to the corynebacteria. Mem. Cornell Univ. Agr. Expt. Stn. no. 366.

312. Ratney, R. S. 1965. The chemistry of the cell walls of *Bacillus anthracis:* the effect of penicillin. Biochim. Biophys. Acta **101**:1-5.
313. Reissig, J. L., J. L. Strominger, and L. F. Leloir. 1955. A modified colorimetric method for the estimation of N-acetylamino sugars. J. Biol. Chem. **217**:959-966.
314. Reuter, G. 1963/64. Vergleichende Untersuchungen über die Bifidus-Flora im Säuglings- und Erwachsenenstuhl zugleich ein Beitrag zur Systematisierung und Nomenklatur der Bifiduskeime. Zentrabl. Bakteriol. Infektionskr. Hyg. Abl. 1, Orig. **191**:486-506.
315. Rhuland, L. E., E. Work, R. F. Denman, and D. S. Hoare. 1955. The behaviour of the isomers of α, ϵ-diaminopimelic acid on paper chromatograms. J. Amer. Chem. Soc. **77**:4844-4846.
316. Roberts, W. S. L., J. F. Petit, and J. L. Strominger. 1968. Biosynthesis of the peptidoglycan of bacterial cell walls. VIII. Specificity in the utilization of L-alanyl transfer ribonucleic acid for interpeptide bridge synthesis in *Arthrobacter crystallopoietes*. J. Biol. Chem. **243**:768-773.
317. Roberts, W. S. L., J. S. Strominger, and D. Söll. 1968. Biosyntheis of the peptidoglycan of bacterial cell walls. VI. Incorporation of L-threonine into interpeptide bridges in *Micrococcus roseus*. J. Biol. Chem. **243**:749-757.
318. Robinson, K. 1966. Some observations on the taxonomy of the genus *Microbacterium*. I. Cultural and physiological reactions and heat resistance. J. Appl. Bact. **29**:607-615.
319. Robinson, K. 1966. Some observations on the taxonomy of the genus *Microbacterium*. II. Cell wall analysis, gel electrophoresis and serology. J. Appl. Bacteriol. **29**:616-624.
320. Robinson, K. 1968. The use of cell wall analysis and gel electrophoresis for the identification of coryneform bacteria. *In* B. M. Gibbs and D. A. Shapton (ed.), Identification methods for microbiologists, part B, p. 88-92. Academic Press Inc., New York.
321. Rogers, H. J., and H. R. Perkins. 1959. Cell wall mucopeptides of *Staphylococcus aureus* and *Micrococcus lysodeikticus*. Nature (London) **184**:520-524.
322. Rogers, H. J., and H. R. Perkins. 1968. Cell walls and membranes. Spon's Biochemical Monographs. E. and F. N. Spon Ltd., London.
323. Rosenthal, S., and N. Sharon. 1964. The use of sephadex G-25 for the isolation of nucleotide sugar derivatives from *Micrococcus lysodeikticus*. Biochim. Biophys. Acta **83**:378-380.
324. Rosypal, S., A. Rosypalova, and J. Horejs. 1966. The classification of micrococci and staphylococci based on their DNA base composition and Adansonian analysis. J. Gen. Microbiol. **44**:281-292.
325. Roth, G. D. 1957. Proteolytic organisms of the carious lesion. Oral Surg., Oral Med., Oral Pathol. **10**:1105-1117.
326. Salton, M. R. J. 1964. The bacterial cell wall. American Elsevier Publishing Co., New York.
327. Salton, M. R. J., and J. G. Pavlik. 1960. Studies on bacterial cell wall. VI. Wall composition and sensitivity to lysozyme. Biochim. Biophys. Acta **39**:398-407.
328. Scardovi, V., and L. D. Trovatelli. 1965. The fructose-6-phosphate shunt as a peculiar pattern of hexose degradation in the genus *Bifidobacterium*. Ann. Microbiol. **XV**:19-29.
329. Scardovi, V., and L. D. Trovatelli. 1969. New species of bifid bacteria from *Apis mellifica* L. and *Apis indica* F. A contribution to the taxonomy and biochemistry of the genus *Bifidobacterium*. Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. 1 **123**:64-88.
330. Scardovi, V., L. D. Trovatelli, F. Crociani, and E. Sgorbati. 1969. Bifid bacteria in bovine rumen. Arch. Mikrobiol. **68**:278-294.
331. Scardovi, V., L. D. Trovatelli, G. Zani, F. Crociani, and D. Matteuzzi. 1971. Deoxyribonucleic acid homology relationships among species of the genus *Bifidobacterium*. Int. J. Syst. Bacteriol. **21**:276-294.
332. Schefferle, H. E. 1966. Coryneform bacteria in poultry deep litter. J. Appl. Bacteriol. **29**:147-160.
333. Schleifer, K. H. 1969. Die Murein (Peptidoglycan)-typen bei grampositiven Bakterien. Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. 1, Orig. **212**:443-451.
334. Schleifer, K. H. 1969. Substrate dependent modifications of the amino acid sequence of the murein of staphylococci. J. Gen. Microbiol. **57**:XIV.
335. Schleifer, K. H. 1970. Die Mureintypen in der Gattung *Microbacterium*. Arch. Mikrobiol. **71**:271-282.
335a. Schleifer, K. H. 1972. Chemical composition of staphylococcal cell walls. *In* J. Jeljaszewicz (ed.), II. Intern. Symp. on Staphylococci and Staphylococcal Infections, S. Karger, Basel.
336. Schleifer, K. H., and O. Kandler. 1967. Zur chemischen Zusammensetzung der Zellwand der Streptokokken. I. Die Aminosäuresequenz des Mureins von *Str. thermophilus* und *Str. faecalis*. Arch. Mikrobiol. **57**:335-364.
337. Schleifer, K. H., and O. Kandler. 1967. Zur chemischen Zusammensetzung der Zellwand der Streptokokken. II. Die Aminosäuresequenz des Mureins von *Streptococcus lactis* und *cremoris*. Arch. Mikrobiol. **57**:365-381.
338. Schleifer, K. H., and O. Kandler. 1967. *Micrococcus lysodeikticus:* a new type of crosslinkage of the murein. Biochem. Biophys. Res. Commun. **28**:965-971.
339. Schleifer, K. H., and O. Kandler. 1968. Zur chemischen Zusammensetzung der Zellwand der Streptokokken. III. Die Aminosäuresequenz eines glycinhaltigen Mureins aus *Pep-*

tostreptococcus evolutus (Prevot) Smith. Arch. Mikrobiol. **61**:292-301.
340. Schleifer, K. H., and O. Kandler. 1970. Amino acid sequence of the murein of *Planococcus* and other *Micrococcaceae*. J. Bacteriol. **103**:387-392.
341. Schleifer, K. H., R. Plapp, and O. Kandler. 1967. The amino acid sequence of a glycine-containing cell wall precursor of *Microbacterium lacticum*. Biochem. Biophys. Res. Commun. **26**:492-496.
342. Schleifer, K. H., R. Plapp, and O. Kandler. 1967. Identification of threo-3-hydroxyglutamic acid in the cell wall of *Microbacterium lacticum*. Biochem. Biophys. Res. Commun. **28**:566-570.
343. Schleifer, K. H., R. Plapp, and O. Kandler. 1968. Die Aminosäuresequenz des Mureins von *Microbacterium lacticum*. Biochim. Biophys. Acta **154**:573-582.
344. Schleifer, K. H., M. Ried, and O. Kandler. 1968. Die Aminosäuresequenz des Mureins von *Staphylococcus epidermidis* (Winslow and Winslow) Evans, Stamm 66. Arch. Mikrobiol. **62**:198-208.
345. Schleifer, K. H., R. Plapp, and O. Kandler. 1968. Glycine as crosslinking bridge in the L,L-diaminopimelic acid containing murein of *Propionibacterium petersonii*. Fed. Eur. Biochem. Soc. Lett. **1**:287-289.
346. Schleifer, K. H., L. Huss, und O. Kandler. 1969. Die Beeinflussung der Aminosäuresequenz des serinhaltigen Mureins von *Staphylococcus epidermidis* Stamm 24 durch die Nährbodenzusammensetzung., Arch. Mikrobiol. **68**:387-404.
347. Schmidt, G., B. Jann, and K. Jann. 1969. Immunochemistry of R lipopolysaccharides of *Escherichia coli*. I. Different core regions in the lipopolysaccharides of O group 8. Eur. J. Biochem. **10**:501-510.
348. Schmidt, G., I. Fromme, and H. Mayer. 1970. Immunochemical studies on core lipopolysaccharides of *Enterobacteriaceae* of different genera. Eur. J. Biochem. **14**:357-366.
349. Schocher, A. J., S. T. Bayley, and R. W. Watson. 1962. Composition of purified mucopeptide from the wall of *Aerobacter cloacae*. Can. J. Microbiol. **8**:89-98.
350. Schultes, L. M., and J. B. Evans. 1970. DNA hybridization among aerococci and related cocci. Pub. Fac. Sci. Univ. J.E. Purkyne Brno **47**:127-129.
351. Schultes, L. M., and J. B. Evans. 1971. Deoxyribonucleic acid homology of *Aerococcus viridans*. Int. J. Syst. Bacteriol. **21**:207-209.
352. Schuytema, E. C., H. L. Glenn, and C. C. Doughty. 1969. Relative rates of lysis of staphylococcal cell walls by lytic enzymes from various bacteriophage types. J. Bacteriol. **98**:920-923.
353. Senn, M., T. Ioneda, J. Pudles, and E. Lederer. 1967. Spectrométrie de masse de glycolipides. I. Structure du "cord factor" de *Corynebacterium diphtheriae*. Eur. J. Biochem. **1**:353-356.
354. Sharon, N. 1969. The bacterial cell wall. Sci. Amer. **220**:92-98.
355. Sharpe, M. E. 1970. Cell wall and cell membrane antigens in the classification of lactobacilli. Int. J. Syst. Bacteriol. **20**:519-533.
356. Shockman, G. D., J. S. Thompson, and M. J. Conover. 1965. Replacement of lysine by hydroxylysine and its effects on cell lysis in *Streptococcus faecalis*. J. Bacteriol. **90**:575-588.
357. Silvestri, L. G., and L. R. Hill. 1965. Agreement between deoxyribonucleic acid base composition and taxometric classification of gram-positive cocci. J. Bacteriol. **90**:136-140.
358. Slade, H. D., and W. C. Slamp. 1962. Cell wall composition and the grouping antigens of streptococci. J. Bacteriol. **84**:345-351.
359. Smith, W. G., and L. M. Henderson. 1964. Relationships of lysine and hydroxylysine in *Streptococcus faecalis* and *Leuconostoc mesenteroides*. J. Biol. Chem. **239**:1867-1871.
360. Soprey, P., and H. D. Slade. 1971. Chemical structure and immunological specificity of the streptococcal group E cell wall polysaccharide antigen. Infect. Immunity **3**:653-658.
361. Starr, M. P., J. E. Weiss, H. P. Klein, and C. B. Sesselman. 1943. Growth of phytopathogenic bacteria in synthetic asparagine medium. Phytopathology **33**:314-318.
362. Staudenbauer, W. 1968. D-aspartic acid incorporation into interpeptide bridges in bacterial cell wall. Fed. Proc. **27**:294.
363. Stoeckenius, W., and R. Rowen. 1967. A morphological study of *Halobacterium halobium* and its lysis in media of low salt concentrations. J. Cell. Biol. **34**:365-393.
364. Strange, R. E., and F. A. Dark. 1956. An unidentified amino-sugar present in cell walls and spores of various bacteria. Nature (London) **177**:186-188.
365. Strange, R. E., and L. H. Kent. 1959. The isolation, characterization and chemical synthesis of muramic acid. Biochem. J. **71**:333-339.
366. Strominger, J. L. 1962. Biosynthesis of bacterial cell walls. Fed. Proc. **21**:134-143.
367. Strominger, J. L., and J. M. Ghuysen. 1967. Mechanisms of enzymatic bacteriolysis. Science **156**:213-221.
368. Strominger, J. L., J. T. Park, and R. E. Thompson. 1959. Composition of the cell wall of *Staphylococcus aureus:* its relation to the mechanism of action of penicillin. J. Biol. Chem. **234**:3263-3268.
369. Strominger, J. L., K. Izaki, M. Matsuhashi, and D. J. Tipper. 1967. Peptidoglycan transpeptidase and D-alanine carboxypeptidase. Penicillin-sensitive enzymatic reactions. Fed. Proc. **26**:9-22.
370. Strominger, J. L., P. M. Blumberg, H. Suginaka,

J. Umbreit, and G. G. Wickus. 1971. How penicillin kills bacteria: progress and problems. Proc. Roy. Soc. London, Ser. B **179**:369-383.
371. Subcommittee on Taxonomy of Staphylococci and Micrococci, 1965. Minutes of first meeting. Int. Bull. Bacteriol. Nomencl. **15**:107-108.
372. Sukapure, R. S., M. P. Lechevalier, H. Reber, M. L. Higgins, H. A. Lechevalier, and H. Prauser. 1970. Motile nocardoid *Actinomycetales*. Appl. Microbiol. **19**:527-533.
373. Suput, J., M. P. Lechevalier, and H. A. Lechevalier. 1967. Chemical composition of variants of aerobic antinomycetes. Appl. Microbiol. **15**:1356-1361.
374. Sutow, A. B., and N. E. Welker. 1967. Chemical composition of the cell walls of *Bacillus stearothermophilus*. J. Bacteriol. **93**:1452-1457.
375. Szaniszlo, P. J., and H. Gooder. 1967. Cell wall composition in relation to the taxonomy of some *Actinoplanaceae*. J. Bacteriol. **94**: 2037-2047.
376. Takumi, K., and T. Kawata. 1970. Chemical composition of the cell walls of *Clostridium botulinum* type A. Jap. J. Microbiol. **14**:57-63.
377. Taylor, A., and M. Stepien. 1971. Similarity of *Salmonella typhi* and *Escherichia coli* mureins. Acta Biochem. Polonica **18**:243-248.
378. Tempest, D. W., J. W. Dicks, and D. C. Ellwood. 1968. Influence of growth condition on the concentration of potassium in *Bacillus subtilis* var. *niger* and its possible relationship to cellular ribonucleic acid, teichoic acid and teichuronic acid. Biochem. J. **106**:237-243.
379. Tepper, B. S., J. A. Hayashi, and S. S. Barkulis. 1960. Studies on streptococcal cell walls. V. Amino acid composition of cell walls of virulent and avirulent group A hemolytic streptococci. J. Bacteriol. **79**:33-38.
380. Tinelli, R. 1966. Etude de la composition du glycopeotide des parois de bactéries grampositives par une microtechnique de chromatographie sur couche mince. Bull. Soc. Chim. Biol. **48**:182-185.
381. Tinelli, R. 1968. Le glycopeotide des parois bactériennes. Bull. Inst. Pasteur **66**:2507-2538.
382. Tinelli, R., and J. Pillot. 1966. Etude de la composition du glycopeptide de *Treponema reiteri*. C. R. Acad. Sci. **263**:739-741.
383. Tinelli, R., M. Shilo, M. Laurent, and J. M. Ghuysen. 1970. De la présence d'un glycopeotide dans la parois de *Bdellovibrio bacteriovorus*. C. R. Acad. Sci. **270**:2600-2602.
384. Tipper, D. J. 1968. Alkali-catalyzed elimination of D-lactic acid from muramic acid and its derivatives and the determination of muramic acid. Biochemistry **7**:1441-1449.
385. Tipper, D. J. 1969. Structures of the cell wall peptidoglycans of *Staphylococcus epidermidis* Texas 26 and *Staphylococcus aureus* Copenhagen. II. Structure of neutral and basic peptides from hydrolysis with the *Myxobacter* AL-1 peptidase. Biochemistry **8**:2192-2202.
386. Tipper, D. J. 1970. Structure and function of peptidoglycans. Int. J. Syst. Bacteriol. **20**:361-377.
387. Tipper, D. J., and M. F. Berman. 1969. Structures of the cell wall peptidoglycans of *Staphylococcus epidermidis* Texas 26 and *Staphylococcus aures* Copenhagen. I. Chain length and average sequence of cross-bridge peptides. Biochemistry **8**:2183-2192.
388. Tipper, D. J., and J. L. Strominger. 1965. Mechanism of action of penicllins: a proposal based on their structural similarity to acyl-D-alanyl-D-alanine. Proc. Nat. Acad. Sci. U.S.A. **54**:1133-1141.
389. Tipper, D. J., W. Katz, J. L. Strominger, and J. M. Ghuysen. 1967. Substituents on the α-carboxyl group of D-glutamic acid in the peptidoglycan of several bacterial cell walls. Biochemistry **6**:921-929.
390. Tipper, D. J., J. L. Strominger, and J. C. Ensign. 1967. Structure of the cell wall of *Staphylococcus aureus*, strain Copenhagen. VII. Mode of action of the bacteriolytic peptidase from *Myxobacter* and the isolation of intact cell wall polysaccharides. Biochemistry **6**:906-920.
391. Toennies, G., B. Bakay, and G. D. Shockman. 1959. Bacterial composition and growth phase. J. Biol. Chem. **234**:3269-3275.
392. Tornabene, T. G., S. J. Morrison, and W. E. Kloos. 1970. Aliphatic hydrocarbon contents of various members of the family *Micrococcaceae*. Lipids **5**:929-37.
393. Ushijima, T. 1970. Morphology and chemistry of the bacterial cell wall. 1. The location of mucopeptide in the cell wall of *Bacteroides convexus* and its chemical composition. Jap. J. Microbiol. **14**:15-25.
394. Van Heijenoort, J., L. Elbaz, P. Dezelee, J. F. Petit, E. Bricas, and J. M. Ghuysen. 1969. Structure of the meso-diaminopimelic acid containing peptidoglycans in *Escherichia coli* B and *Bacillus megaterium* KM. Biochemistry **8**:207-211.
395. Veerkamp, J. H. 1971. The structure of the cell wall peptidoglycan of Bifidobacterium bifidum var. *pennsylvanicus*. Arch. Biochem. Biophys. **143**:204-211.
396. Venkataraman, R., and A. Sreenivasan. 1956. Red halophilic bacteria—the identity of some wellknown species. Proc. Indian Acad. Sci. B **43**:264-270.
397. Verma, J. P., and H. H. Martin. 1967. Über die Oberflächenstruktur von Myxobakterien. I. Chemie und Morphologie der Zellwände von *Cytophaga hutchinsonii* und *Sporocytophaga myxococcoides*. Arch. Mikrobiol. **59**:355-380.
398. Vilkas, E., J. C. Massot, and E. Zissmann. 1970. Etude des parois d'une souche de *Micromonospora*. I. Isolement d'une phosphate de glucosamine. Fed. Eur. Biochem. Soc. Lett.

7:77-79.
399. Volk, W. A. 1966. Cell wall lipopolysaccharides from *Xanthomonas spec.* J. Bacteriol. **91:** 39-42.
400. Wang, W. S., and D. G. Lundgren. 1968. Peptidoglycan of a chemolithotrophic bacterium, *Ferrobacillus ferrooxidans.* J. Bacteriol. **95:**1851-1856.
401. Warth, A. W., and J. L. Strominger. 1969. Structure of the peptidoglycan of bacterial spores: occurrence of the lactam of muramic acid. Proc. Nat. Acad. Sci. U.S.A. **64:**528-535.
402. Warth, A. D., and J. L. Strominger. 1971. Structure of the peptidoglycan from vegetative cell walls of *Bacillus subtilis.* Biochemistry **10:**4349-4358.
403. Weidel, W., and H. Pelzer. 1964. Bagshaped macromolecules: a new outlook on bacterial cell walls. Advan. Enzymol. **26:**193-232.
404. Weidel, W., and J. Primosigh. 1957. Die grameinsame Wurzel der Lyse von *Escherichia coli* durch Penicillin oder durch Phagen. Z. Naturforsch. **12b:**421-427.
405. Weidel, W., H. Frank, and W. Leutgeb. 1963. Autolytic enzymes as a source of error in the preparation and study of gram-negative cell walls. J. Gen. Microbiol. **30:**127-130.
406. Weiss, N., R. Plapp, and O. Kandler. 1967. Die Aminosäuresequenz des DAP haltigen Mureins von *Lactobacillus plantarum* und *Lactobacillus inulinus.* Arch. Mikrobiol. **58:**313-323.
407. Welby-Gieusse, M., M. A. Lanéelle, and J. Asselineau. 1970. Structure des acides corynomycoliques de *Corynebacterium hofmanii* et leur implication biogénétique. Eur. J. Biochem. **13:**164-167.
408. Werner, H. 1966. The gram-positive nonsporing anaerobic bacteria of the human intestine with particular reference to the corynebacteria and bifidobacteria. J. Appl. Bacteriol. **29:**138-146.
409. Werner, H. 1967. Die Bedeutung von DNS-Basen-Bestimmung für die bakteriologische Systematik. Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. 1, Orig. **205:**210-218.
410. Werner, H., and S. Mann. 1968. Chemische Analyse der Zellwand von *Corynebacterium acnes* und *Corynebacterium parvum.* Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. 1, Orig. **206:**486-499.
411. Werner, H., F. Gasser, and M. Sebald. 1966. DNS-Basenbestimmungen an 28 Bifidus-Stämmen und an Stämmen morphologisch ähnlichen Gattungen. Zentrabl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. 1, Orig. **198:**504-516.
412. Westphal, O., F. Kauffmann, O. Lüderitz, and H. Stierlin. 1960. Immunchemie der O-Antigene von *Enterobacteriaceae.* III. Analyse der Zuckerbausteine kreuzreagierender *Salmonella-* und *Escherichia*-O-Antigene. Zentrabl. Bakteriol. Parasitenk. Infectionskr. Abt. 1. Orig. **179:**336-342.
413. Wheat, R. W., and J. M. Ghuysen. 1971. Occurrence of glucomuramic acid in gram-positive bacteria. J. Bacteriol. **105:**1219-1221.
414. Wheat, R. W., S. Kulkarni, A. Cosmatos, E. Scheer, and R. Steele. 1969. Galactomuramic acid. Chemical synthesis, properties, assay and survey in several bacterial specimens. J. Biol. Chem. **244:**4921-4928.
415. White, D., M. Dworkin, and D. J. Tipper. 1968. Peptidoglycan of *Myxococcus xanthus:* structure and relation to morphogenesis. J. Bacteriol. **95:**2186-2197.
416. White, P. J. 1968. A comparison of the cell walls of *Pediococcus cerevisiae* and of a substrain that requires methicillin for growth. J. Gen. Microbiol. **50:**107-120.
417. Whittenbury, R. 1966. A study of the genus *Leuconostoc.* Arch. Mikrobiol. **53:**317-327.
418. Whiteside, T. C., A. J. De Siervo, and M. R. J. Salton. 1971. Use of antibody to membrane adenosine triphosphatase in the study of bacterial relationships. J. Bacteriol. **105:**957-967.
419. Whitney, J. G., and E. A. Grula. 1968. A major attachment site of D-serine in the cell wall mucopeptide of *Micrococcus lysodeikticus.* Biochim. Biophys. Acta **158:**124-129.
420. Wietzerbin-Falszpan, J., B. C. Das, I. Azuma, A. Adam, J. F. Petit, and E. Lederer. 1970. Isolation and mass spectrophotometric identification of the peptide subunits of mycobacterial cell walls. Biochem. Biophys. Res. Commun. **40:**57-63.
421. Wickus, G., and J. L. Strominger. 1971. A penicillin-sensitive transpeptidase in a particulate enzyme preparation from a gram-positive organism. Fed. Proc. **30:**1174.
422. Williams, R. A. D., and S. Sadler. 1971. Electrophoresis of glucose-6-phosphate dehydrogenase, cell wall composition, and the taxonomy of heterofermentative lactobacilli. J. Gen. Microbiol. **65:**351-358.
423. Williams, S. T., F. L. Davies, and T. Cross. 1968. Identification of genera of *Actinomycetales,* p. 111-124. *In* B. M. Gibbs and D. A. Shapton (ed.), Identification methods for microbiologists, part B. Academic Press Inc., New York.
423a. Winter, A. J., W. Katz, and H. H. Martin. 1971. Murein (peptidoglycan) structure of *Vibrio fetus:* comparison of a venereal and intestinal strain. Biochim. Biophys. Acta **244:**58-64.
424. Wolin, M. J., A. R. Archibald, and J. Baddiley. 1966. Changes in wall teichoic acid resulting from mutations of *Staphylococcus aureus.* Nature (London) **209:**484-486.
425. Work, E. 1957. Biochemistry of the bacterial cell wall. Nature (London) **179:**841-847.
426. Work, E. 1969. Biochemistry of bacterial cell walls. Lab. Pract. **18:**831-838.
427. Work, E. 1970. The distribution of diamino acids in cell walls and its significance in bacterial taxonomy. Int. J. Syst. Bacteriol. **20:**425-433.
428. Yamaguchi, T. 1965. Comparison of the cell-wall composition of morphologically distinct ac-

tinomycetes. J. Bacteriol. **89**:444–453.

429. Yamada, K., and K. Komagata. 1970. Taxonomic studies on coryneform bacteria. II. Principle amino acids in the cell wall and their taxonomic significance. J. Gen. Appl. Microbiol. **16**:103–113.
430. Young, F. E. 1965. Variation in the chemical composition of the cell walls of *Bacillus subtilis* during growth in different media. Nature (London) **207**:104–105.
431. Young, F. E. 1966. Fractionation and partial characterization of the products of autolysis of cell walls of *Bacillus subtilis*. J. Bacteriol. **92**:839–846.
432. Zierdt, C. H., C. Webster, and W. S. Rude. 1968. Study of the anaerobic corynbacteria. Int. J. Syst. Bacteriol. **18**:33–47.
433. Zygmunt, W. A., H. P. Browder, and P. A. Tavormina. 1967. Lytic action of lysostaphin on susceptible and resistant strains of *Staphylococcus aureus*. Can. J. Microbiol. **13**:845–853.
434. Zygmunt, W. A., H. P. Browder, and P. A. Tavormina. 1968. Susceptibility of coagulase negative staphylococci to lysostaphin and other antibiotics. Appl. Microbiol. **16**:1168–1173.